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PROBLEMS OF ANTI-BACTERIOLOGICAL DEFENSE:

ACCORDING TO DATA FROM FOREIGN LITERATURE

by P. F. Zdrodovskiy

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## FOREWORD

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PROBLEMS OF ANTI-BACTERIOLOGICAL DEFENSE:  
ACCORDING TO DATA FROM FOREIGN LITERATURE

[Following is a translation of a book under joint authorship and edited by P. F. Zdrodovskiy entitled Voprosy Protivo-Bakteriologicheskoy Zashchity (English version above), Moscow, 1960, pp 1 - 320.]

ANNOTATION

This monograph is a generalization of information which has been published abroad in the literature treating the problem of the bacteriological weapon and defense measures against it.

The book is aimed at acquainting medical workers with the characteristics of the bacteriological weapon, as well as the means and methods of providing defense against it.

This book sets forth the principles of organizing both individual and collective anti-bacteriological defense, as found in the foreign literature.

"We share the concern of scientists who correctly point out that the employment of this weapon can entail consequences that are not less horrible than those produced by the use of atomic and hydrogen weapons. It was not merely by chance that the chemical and bacteriological weapons were outlawed for warfare by the decision of the nations, as expressed in international



agreement in the Gen. Protocol in 1925.

The USSR is, as it is well known, a decidedly strong advocate of prohibition of all forms of weapons of mass destruction, including both nuclear and the chemical and bacteriological weapons. We are of the opinion that their use violates the principles of humanity, the standards of international law and the conscience of the peoples.

N. S. Krushchëv

(Excerpt from a message sent to the Pugwash scientific conference in August 1959.)

#### TABLE OF CONTENTS

Preface . . . . .	page 4
Chapter I. Brief Historical Sketch. Lebedinskiy, V. A. . . . .	6
Chapter II. Information on the Bacteriological Weapon. Gapochko, K. G.; Garin, N. S.; Lebe- dinskiy, V. A.; Filippenko, A. I. . . . .	35
Chapter III. Some Problems in the Mass Procure- ment of Biological Agents. Filippenko, A. I. . . . .	74
Chapter IV. Principles of Selection and Possible Agents of Bacteriological Warfare. Garin, N.S. . . . .	117
Chapter V. Basic Means of Anti-Bacteriological Defense. . . . .	187
Organization of Anti-Bacteriological Civil Defense in the United States of America. Gapochko, K. G. . . . .	187
Principles of Detecting the Biological Infectious Agents. Lebedinskiy, V. A. . . . .	200
Individual Protection against the Bacterio- logical Weapon. Burgasov, P. N.; Gapochko, K.G. . . . .	253

Means of Individual and Collective Non-specific Defense. Filippenko, A. I. . .	309
Several Modern Means and Methods of Disinfection. Filippenko, A. I. . . . .	319
Organization of Anti-Bacteriological Defense Measures. Gapochko, K. G. . .	340

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## PREFACE

We are experiencing an international situation where, because we are not pursuing the arms race of the capitalistic countries, we are forced to concentrate on the problem of defending ourselves from the bacteriological weapon, the possible application of which has been stated repeatedly and vigorously for the last decade in the periodical literature of some capitalistic countries. As evidence of this it is sufficient to refer to an article published in the journal, "Public Health Reports", in the USA during 1957, where it was stated, *inter alia*, "...work on the study and development of the biological weapon continues even to this day; the Department of Defense is officially engaged in it. The responsibility for the fulfillment of the program on the biological weapon is put on the Chemical Warfare Service. The scientific investigative work on the biological weapon is primarily centralized at Fort Detrick (Frederick, Maryland). In addition, a close link is sustained with other federal organizations committed to expedite the consolidation of defense resources.

"The fact that the work on the study and development of the biological weapon is conducted in institutes

created especially for this purpose can be considered an indication of the might of this weapon"<sup>1</sup>. It must be pointed out that the author of the cited article is LeRoy Fothergill, a scientific consultant with this same Chemical Warfare Service, the staff of which is engaged in a study and preparation of the biological (bacteriological) weapon.

The appearance of such statements in the literature of the capitalistic countries stimulates the study and systematization of all the material published abroad on bacteriological warfare in order to form a definite idea about this problem in all of its aspects.

Consequently, a voluminous amount of literary material, which thoroughly exposed the problem of bacteriological warfare and measures of defense against it, was collected and studied. The book, which is called to the attention of the reader, is just a literary digest of the indicated data.

There is scarcely a need to prove the indispensability and the timely publication of this book, because the Soviet medical profession must certainly become acquainted in detail with the current concept of the question of bacteriological warfare and defense against the bacteriological weapon abroad, all the more so since there are no similar publications in our own medical literature.

P. Zdrodovskiy  
Active member of  
Academy of Medical Sciences USSR

<sup>1</sup>Fothergill, LeRoy D., "Biological Warfare and Its Defense", Public Health Reports, 1957, 72, 10, 865 - 871.

## CHAPTER I

### BRIEF HISTORICAL SKETCH

The bacteriologic-al weapons are comprised of pathogenic microorganisms, toxic products of their life activities (toxins), insect pests, and some synthetic substances (so-called antihormones or herbicides) meant for agricultural animals, grains, and crops of the adversary, and also the agents of their application.

The application of the enumerated infective agents for the achievement of military purposes is termed "bacteriological warfare".

The terms "bacteriological weapon" and "bacteriological warfare", as it already appears from the terminology itself, now have to be conceded as somewhat out-dated. Actually, if originally the infective agents considered for use were only microorganisms belonging to the group of bacteria (glanders and anthrax bacteria), then at present, judging by the available literary data on bacteriological warfare, some other microorganisms, particularly rickettsiae, viruses, and fungi, could find application.

The meaning of these terms becomes very inaccurate when they are extended to insect pests, which can also be used for the contamination of agricultural plants.

It must be admitted that more precise terms and ones, which more clearly reflect the contents of the specific object, are "biological weapon" and "biological warfare", and nowadays these have supplanted the terms "bacteriological weapon" and "bacteriological warfare" in the literature and official manuals of foreign countries. However, even they are not clear enough, since herbicides, which at present refer to the biological weapon, are synthetic chemical substances.

In spite of the sentiments expressed above about the fallacy of the existing terminology, in further comments we will nevertheless be forced to use the term "bacteriological weapon", since it is retained in the literature even at the present time.

\* \* \*

A study of the history of warfare both in ancient times and in the not so distant past shows that military activities, as a rule, were accompanied by epidemics. The losses, which armies have borne from infectious diseases, are often considerably higher than those due to the effect of weapons.

Smallpox cut down the troops of Alexander of Macedonia returning from a campaign in India, annihilated the Abyssian army laying siege on Mecca in 571, and infected the Crusaders at the time of the Crusades in the 11<sup>th</sup> to 13<sup>th</sup> centuries (1).

Armies suffered not much less in losses due to epidemics even at a later time. During the siege of Grenada in 1489 17,000 persons in the Spanish army succumbed to typhus - almost six times more than were killed in the battle with the Moors. Typhus wiped out 30,000 French soldiers laying siege to Neapoli in 1528, and the rest of the army, raising the siege, were compelled to retreat (2).

In the period of the Crimean campaign of 1854 - 1856 epidemics of cholera, typhoid fever, and typhus took on such a dangerous character that the fighting efficiency of the armies was notably lowered. In the British army for every 100 persons dying on the field of battle there were 380 persons dying from infectious diseases. In the French armies this ratio was correspondingly expressed in the figures 100:371, and in the Russian - 100:219. The French land forces even before arriving in Crimea lost

10% of the personnel (3,000 sick, 5,000 dead) from cholera. For three months in the English army, operating in the vicinity of Varna, 2,615 persons (87% of the personnel) became sick with cholera. The small Sardinian army lost 1,230 persons from cholera while 12 persons in all fell on the battlefield (3).

The high figures for infectious diseases also characterize the more recent wars at the end of the 19<sup>th</sup> and beginning of the 20<sup>th</sup> century. The incidence of typhoid fever and malaria was extremely high in the armies of the United States of America in the period of the Civil War of 1861 - 1865; typhoid fever afflicted more than 30% of the personnel (4), and the number of malaria cases exceeded 1,200,000 (5).

But, in general, in the second half of the 19<sup>th</sup> century from 10 to 40% of the personnel in the fighting armies was put out of commission from typhoid fever (6).

Advancement in prophylactic and therapeutic measures of infectious diseases along with a higher level of the general sanitary-hygienic state of the troops occasioned some decrease in morbidity in the armies. Even in the First World War the importance of losses from infectious diseases had significantly lessened, which, however, could be partially attributed to the greater effectiveness of the lethal force of the weapon, because morbidity in armies from typhoid fever, typhus, dysentery, and malaria, especially in certain theaters of military action, continued to remain at a relatively high level. In the Russian army in the European theater about 16 cases of dysentery were reported for every 1,000 persons, in Germany - about 24, in the English army in Mesopotamia - 109, in the Dardanelles - 253, in Eastern Africa - 277. Thus, in certain fighting armies dysentery afflicted 25 - 27% of the personnel (7).

The Serbian and Turkish armies suffered acutely from typhus in the period of the First World War.

Malaria was also widespread in the fighting armies. Thus, of 120,000 persons in the French armies, located in the Balkans, 30% were hospitalized with malaria. The English army, operating in the Near East and in Eastern Africa, also bore enormous losses from malaria. For example, in Mesopotamia every tenth soldier was sick with malaria, in Gallipoli and Eastern Africa - every fourth soldier.

A pandemic of viral grippe, breaking out at the end of the war, afflicted not only the population but the troops as well. In the French army, even according to far from complete data, the number of afflicted during the

course of a year consisted of 408,100 persons of whom more than 30,000 died. Even in the Swiss army, which does not engage in military activities and therefore is in a more favorable situation than the fighting armies, about 66% of the personnel were ill (8).

The Second World War also offers examples of what dimensions outbreaks of sickness can assume in armies, which seriously complicate their military action. Thus, the high morbidity rate of tsutsugamushi fever among the English and American troops showed an unfavorable influence on the course of military operations in the Pacific theater of action. Some divisions were literally paralyzed. For example, 6 - 12 days after debarkation of one of the American regiments on the island of Sansapor 400 persons became ill with tsutsugamushi fever. During the second week morbidity reached 900 cases per annum for every 1,000 soldiers. A high percent of morbidity with tsutsugamushi fever was also noted among the English troops located in Burma. The number of afflicted in some sections ran to one-fifth of the total personnel. So in the armies of the English-American alliance in the Pacific theater, according to incomplete data, there were recorded in all about 20,000 afflicted (9):

Profound complications arose during the military operations of the alliance in 1942 in Northern Africa because of epidemic typhus and dysentery which raged in these districts.

Large outbreaks of typhus were prominent in the German armies operating on the Eastern front. These outbreaks were one of the results of a lack of providing the Hitler army with elementary agents of anti-epidemic defense and the absence of a systematic anti-epidemic service. At the end of 1942 in some German armies the number of sick, which was estimated in the thousands, equalled the number of wounded (10).

According to the data of English authors a high morbidity rate from dysentery was noted among the Italian troops in Libya.

Numerous examples are also known from the history of war of how the appearance of epidemics in armies leads to the collapse of certain operations and sometimes even of whole campaigns.

Thus, at the time of the Iranian-Turkish War a cholera epidemic brought about a loss of fighting efficiency in the combat armies, and the battle came to a stop. Outbreaks of cholera have repeatedly brought collapse to the English campaigns in India and the French ones in Northern



Africa. During the Crimean war one French division, attacking in the district of Var a, lost about 2,000 persons from cholera in less than one month. In this division all of the personnel were affected, as a result of which the French had to give up the offensive operation and return to the original position, not coming into contact with the Russian soldiers (3).

In 1859 in Algiers from a French detachment of fifteen thousand 10,000 - 12,000 were afflicted with cholera, which led to the collapse of military actions.

Malaria, infecting the English and French troops operating in Macedonia during the First World War, actually led to the downfall of the Balkan operation of the Alliance. The morbidity took on catastrophic dimensions: merely from July to December 1916 malaria infected more than 60,000 persons. In some battalions located in Salonica malaria cases were reported in 95% of the personnel.

The cited examples, the number of which could be considerably increased, obviously proves the point that epidemics arising in armies during their conduction of military activities did impressively complicate and sometimes also completely disrupted operations.

Epidemics, originating in war time at the rear among the civilian population and disorganizing the activities of industry, transportation, and government, create serious events. During the First World War typhus epidemics flared up in Serbia - less than six months afterward 150,000 persons were dead (11). At the end of the First World War the most severe pandemic of viral grippe enveloped all of the fighting countries and many of the neutral ones. In 1 1/2 years 500 million people had been taken sick; 20 million of them died, which was four times the number killed in the years of the First World War.

From all that has been said, however, the conclusion must not be made that epidemic catastrophes are absolutely a compulsory companion of each war. A well-defined and thorough system of prophylactic measures accompanied by sufficient material facilities, high qualifications and selflessness of workers of the army medical service and public health organs are those factors which can play a decisive role in the realm of preventing outbreaks of infectious diseases, their localization, and salvage of the aftermath. The fallacy of maintaining the inevitability of an epidemic in war time is more dramatically proved by the experience of the Great Patriotic War, during which neither in our armies nor among the population at the rear, in spite of the extraordinary intricacy of the epi-

demiological situation, was there any kind of notable spread of infectious diseases which would in any way prevent the successful conduction of military operations or complicate the work at the rear. In an article about bacteriological warfare some foreign authors have tried to convince the reader that the application of the bacteriological weapon without fail provokes some repercussions which are "catastrophic". As it is seen from the foreign publications, this assertion does not imply that the bacteriological weapon does not represent a serious danger with proper preparation and excellent organization of an anti-epidemic service. In regard to the threatening spread of some infections by the airborne route, one general sanitary and anti-epidemic measure to clear away the after effect of the application of the bacteriological weapon, without a doubt, is not enough. Experience has taught that when the spread of a number of infections (intestinal, cutaneous) can be fairly easily suppressed by performing general sanitary steps and common anti-epidemic measures, this may not be attributed to airborne infections - for example, grippe. It is not difficult to see that the tremendous successes in sanitary and anti-epidemic matters achieved in recent years hardly in any way influences the spread of this disease.

The military and economical potential of the belligerent countries can be weakened not only by epidemics among the troops and population. A few examples are known of how disaster can bring widespread disease to agricultural animals and plants. As an illustration of this, it is sufficient to become acquainted with some figures on cattle plague during individual epizootic cases. Thus, in Rhodesia in one year (1897 - 1898) 1.5 million head of large cattle fell, in the Transvaal in the same period - one million, in Bechuanaland - 1,250,000. At the time of the epizooty, which raged in the Philippines from 1917 to 1927, almost all the cattle died, as a result of which the country was in a very serious economical state.

And an epiphytotic state causes no less destruction. In 1845 - 1847 a potato blight appeared in almost all the western European countries. The destruction caused by this disease was especially high in economically backward Ireland where the potato is one of the principal food products. The loss in the potato crop for two years in a row brought about famine, as a result of which about one million inhabitants of Ireland died and 1.5 million were forced to emigrate (12).

Other similar facts are also known. An outbreak of

a viral disease (dwarfism) in rice, which originated in 1733 on one of the islands of Japan, led to the death of 12,000 persons from starvation.

Extreme complications in supplying the population and army induced an epiphytic state of potato blight during the First World War in Germany. In 1917 Phytophthora destroyed about one-third of the entire potato crop consisting at that time of the greater share of the military ration of the Germans. The worsening of the already poor nutrition reflected markedly on the moral and physical state of the nation.

The widespread occurrence of plant diseases is not only an attribute of history. Today plant diseases also cause great and sometimes even enormous damage to various agricultural seedlings and to the industry connected with the produce. Different forms of mildew, root rot of sprouts and seedlings, and also other diseases of cereal grains (wheat, corn, barley, oats) in the United States of America are the causes of arrears in the amount of grain which would be enough to guarantee a supply to approximately 20 million people for a year (13). Infection of the sugar beet with curly top virus in a number of stricken areas of the USA was responsible for the fact that the sugar industry there completely declined in 1932. In 1935 epiphytic stem rot of wheat in the state of Minnesota was the reason for the loss of 60% of the wheat harvest (14). Devastation no less in scale was also brought to young plants by insects - agricultural pests.

The presented facts allow us to conclude that the appearance in war time of epidemics in the armies and among the civilian population at the rear, especially if epizooty and epiphytoly are associated with them, can to a marked degree undermine the military and economical potential of the country, as this has been repeatedly shown in past wars.

These facts a long time ago attracted some military specialists to the idea of the artificial spread of infectious diseases for the purpose of achieving victory over the adversary.

The first such attempts relate to ancient times. It is known that before our era during a war the corpses of persons who had died from plague or cholera were passed over the walls of besieged cities for this purpose. The uninterred corpses were left where the enemy was supposed to pass, or they were used to contaminate the water source.

During the campaign of Alexander of Macedonia in Middle Asia the inhabitants, trying to stop the invasion

of the foreign army, threw animal corpses into wells along its route (15).

The Tatars, who were surrounding the fortress at Kaffa (at the site of the present Pheodosia), used one such method outside the city of Tana (nowadays Azov). They threw over the wall corpses of their soldiers who had died from the plague. An epidemic flared up among the guards of the fortress, forcing them to give up and flee on ships to Italy. Plague was brought into the ports of Italy and Marseille from where it penetrated into the countries of Central Europe and eventually across Poland into Russia (16).

At the beginning of the 16<sup>th</sup> century suggestions of ways and means to spread infectious diseases in the armies of the enemy could already be found in some tactical handbooks. Such data were discovered, for example, in Tiorakenti di Rononia's tactics, relevant to the 16<sup>th</sup> century, and in the book "Method of Combat" written by the Litovian author, Ziminovich in 1850 (17).

In 1763 the English colonists deliberately spread smallpox among the Indian tribes who inhabited Canada. It is obvious from the extant documents that on the instructions of Governor General Amherst Capital Ecuyer of Colonel Bouquet's command at Fort Pitt transmitted to two Indian chiefs two blankets and a handkerchief which previously had been used by smallpox patients. As a result, a smallpox epidemic broke out among the Indians (18, 19). According to Waksman (20), the conquerors of Pizarro did the same thing.

Bullene (21) attributes to Napoleon the deliberate flooding of the approaches to the surrounded fortress of Mantua, anticipating that malaria would hasten the capitulation of the Italians.

However, the enumerated cases of the use of infectious diseases to combat the enemy are only isolated attempts. The more or less systematic treatment of questions relating to the creation of a bacteriological weapon could begin and begin only on the attainment of a certain level of knowledge about microorganisms and the routes of their dispersion. According to our available data the very first beginnings in this direction originated at the start of the 20<sup>th</sup> century in Germany under the Kaiser. During the First World War Germany made a series of attempts of the military use of bacterial agents, not going, however, outside the framework of sabotage. French General Marsenac (22), the head of the army veterinary service during the First World War, reported one such case based

on authentic facts drawn from official sources. In 1916 the German mission in Bucharest received by diplomatic mail a parcel which later inadvertently fell into the hands of the Roumanian military powers. An examination of its contents, done by the well-known Roumanian bacteriologist, Babes, revealed that ampules found there contained cultures of anthrax and glanders. The instructions attached to the ampules indicated beyond a doubt that the cultures were meant for sabotage infection of large cattle and horses. It read: "In the package there is one ampule for horses and four for large cattle. Use as directed. Each ampule is calculated for the infection of 100 animals. Infect the animals directly through the mouth or, if not possible, mix the contents with the feeding".

There is also widely known the facts about German saboteurs infecting with anthrax and glanders the cattle purchased by the Allied Powers in countries of Latin America. Following this in 1918 the exportation of cattle from Argentina to France was completely halted.

In 1917 the German agents successfully infected with glanders 4,500 mules shipped by the Allied armies from Mesopotamia.

A number of attempts to use bacteriological agents directly at the front are also known. Thus, on the Western front at the location of the French troops a German agent was captured, and upon searching him he was found to be in possession of a culture of glanders organisms. At the order of the Commander-in-Chief of the French Army No. 4376 on 26 March 1917 there was reported to this effect: "In the pre-frontal zone a German agent was apprehended on whom suspicious material was found. He was forced to confess that he had been assigned the mission of inducing an epidemic of glanders among the horses of the French army. A metal tube was found on the man who was arrested, and in it there was a long glass bottle containing a nutrient medium with a glanders culture. A brush was attached to the tube on an iron wire. The German agent had received instructions to use the culture by two methods - to sprinkle it on the feeding intended for the horses or to instill it into the nasal orifice by means of the brush". Three months later the Commander-in-Chief reported a repetition of the episode (23).

An attempt was also made to circulate hoof and mouth disease on French territory. However, soon after cases of sickness appeared even on the territory of the opposite side from where this infection had been seeded (24).

The German command evidently did not propose to be limited to trivial sabotage and planned the military application of bacterial agents on a larger scale. This has been confirmed by innumerable evidence detected on some sections of the front abandoned by retreating German divisions, that is, a large number of ampules with virulent cultures. A high officer of the Kaiser's military-naval fleet, Klotz (25), in the book "The New German War" alludes to the bacteriological attack made in 1918 against the Allied Armies on the western front. This experiment, the consequences of which would have been infinite, was only interrupted on 10 November 1918. "We recall", he wrote, "the experiment undertaken by the army of the former German Crown Prince on the Western front. An armistice put an end to this repulsive crime".

Attempts to spread infectious diseases by German saboteurs were also made in Russia. In 1915 Russian counterespionage apprehended a German agent, Gregorsen, who brought cultures of plague bacteria into Arkhangel'sk via the United States of America. As he disclosed on questioning, these cultures were meant to spread plague among the population of Petrograd. With the arrest of another German saboteur a plan of the assignments to infect and poison the water, water sources, and forage was confiscated (26).

Liepmann (27) reports that at the end of the First World War German fliers discarded contaminated food products over Roumanian cities.

Marsenac (22), referring to an article of the Swedish Doctor Voker [sp. ?], also confirms that at the end of the war the Germans made attempts to spread cholera among the population of the countries opposing Germany.

In spite of defeat in the First World War German militarists did not give up the idea of using the bacteriological weapon in future wars which the vindictive circles planned.

In 1934 the well-known English journalist, Steed (28), published fortuitously obtained secret documents of the steps in an aerial chemical attack of the German military ministry, and these contained data on experiments conducted on the dissemination of bacteria through the air. This material particularly mentioned experiments performed by German agents in Paris and London. The excerpts presented by Steed substantiate the fact that these experiments were for the purpose of revealing the possible infection of the population of these cities by means of distributing the microorganisms by the airborne system.

A culture of *B. prodigiosum* was used as the test organism. The recovered data was verified eventually in Berlin and Hamburg. Several experiments on the airborne dispersion of microorganisms were conducted the suburbs of Paris.

In the 30's, according to the evidence of Alouz (25), a number of inventions were patented in Germany which could be applied for the achievement of a bacteriological attack. Specifically, Yunkers, under the innocent title, "Sprays used to combat insect pests", patented a method which could be successfully used for the spraying of poisonous substances and pathogenic bacteria from an airplane.

At the beginning of the Second World War there was a whole group of scientific-research institutes in Germany working on types of the bacteriological weapon, and the largest in the country was the bacteriological institute of Robert Koch, which from the moment of Hitler's rise to power went under the control of the War Ministry (27, 29, 30). A large laboratory near Berlin worked on the problem of dispersing the pathogenic bacteria. There, in particular, were made models of bacteriological aviation bombs holding from 50 g to five kg of bacterial suspension (so-called "baby bomb").

That the German command planned to use the bacteriological weapon in imminent war actions was affirmed by the fact that for several months before the occupation of Norway, Holland, and France by the Germans, as this has been reported in an edited statement of an English pharmaceutical journal (31), they had purchased abroad the necessary material for the preparation of the nutrient media. The size of the purchases proved that an estimate had been made for mass production. Another article, which had been bought in unusually large quantities, was lycopodium. The author, who cited these facts, expressed the belief that the lycopodium might have been used as an adsorbent of the bacteria intended for airborne dissemination.

During military actions on the Eastern front the German Fascist command tried several times to install bacteriological sabotage. Although these attempts were relatively primitive, nevertheless they proved that the Soviet armies were encountering an enemy who was ready to apply any means. The first sabotage was exposed in the winter of 1942 when the escape of a group of Red Army soldiers, louse-infested and sick with typhus, was especially organized from a Fascist concentration camp for military prisoners. They escaped unchallenged and passed through

the front lines to the territory occupied by our divisions. These people were quickly detected and hospitalized, but in their wake there sprang up several cases of sickness among the personnel coming in contact with them (10.32)

Another bacteriological sabotage was planned by the German Fascist command on quite a broader scale. In 1941 the attacking division on one of the Belorussian fronts discovered near the town of Ozarichi in Poleskaya Oblast, not far from the front border of the German side, three concentration camps where about 33,000 persons were held under horrible conditions. A typhus epidemic raged among the imprisoned. It was established by the High Inquiry of the government commission (33) that these camps had been erected for the purpose of spreading typhus among the personnel of the Soviet army and the civilian population. Typhus patients were carried from various districts of Poleskaya, Gomel'skaya, Minskaya, and other temporarily occupied oblasts of BSSR into the camps and deliberately mixed with the healthy prisoners. The plan evolved from the fact that the movement from camps in rear districts of patients, who were evacuated with the advance of the Soviet army, caused the emergence there of a typhus epidemic, and the direct contact of our personnel with them brought about the infection of the fighting contingents. However, the timely applied procedures of the medical service of the Soviet army helped us to prevent the wide spread of typhus in the armies as well as among the civilian population.

There are also data on the preparation of the German-Fascist command to apply the bacteriological weapon on the Western front, too. The Office of Strategic Services of the Anglo-American Alliance in 1943 reported that the bombardment of the British Isles was planned by rocket missiles bearing bacterial agents (34).

The screen of strict secrecy, which covered the preparations of Hitler Germany for bacteriological warfare, was raised a little at the Nuremberg Trials by the testimony of a former chief of the medical service of the German army, Major General Schreiber (35). As it appears from his testimony, the drastic intensification in the preparations for applying the bacteriological weapon pertained to the period of defeat of the Fascist armies near Stalingrad, when the German command tried desperately to explore some new way of carrying on warfare which they hoped would help decide the outcome of the war in favor of Germany.

In 1943 the chief of staff of the general office of the German military forces called a special meeting at



which he announced that as a result of the state of affairs it was necessary to consider the application of the bacteriological weapon. At the time of the conference a special committee was appointed to handle all the activities in the sphere of bacteriological warfare. Members of the committee included representatives of the scientific sections of the military ground forces, the division of arms and research, military-sanitary and military-veterinary control, staff officers of the military air force, and some civilian experts (zoologists and botanists). Former Deputy Chief Blom of the Imperial Medical Service was appointed director of the operation.

In addition to the already existing organizations, in Poznan still another institute was created where experiments were performed on the dissemination of pathogenic microorganisms by plane, and investigations were also carried out on the potential use of pests of agricultural plants for military purposes.

In 1945, when the advancing sector of the Soviet army was approaching Poznan, the institute was liquidated, and its personnel, part of the equipment, and cultures were evacuated to Tübingen where the interrupted activities were continued in hastily constructed buildings.

The Nuremberg Tribunal also produced evidence attesting to the fact that active operations on the bacteriological weapon were also in progress in Germany during the Second World War in an institute located not far from Saxonburg. One of the primary problems under consideration was the mass cultivation of plague bacteria. The president of the Robert Koch Institute, Professor Hildebrandt, directed the program.

There was no doubt that on the eve of defeat the Fascist leaders, trying at any cost to remain in power, would not stop before applying the bacteriological weapon on a broad scale, in spite of the fact that this was a danger to which in this instance the population of Germany itself might be subjected. However, the headlong advance of the Soviet army warded off the horrible catastrophe prepared for the peoples of Europe.

Intensive development of the bacteriological weapon was also carried on by imperialistic Japan in the period of the 30's and 40's.

We gathered the data presented in this section from the proceedings of the Khaparovsk trials concerning the former officer of the Japanese army who was charged with the preparation and application of the bacteriological weapon (36) and also from the book by the former mil-

itary officer of the Japanese army, Akiyama Kirosi (37) who served in 1945 in one of the Japanese institutes (detachment no. 731) which was engaged in preparation for bacteriological warfare.

The idea of using pathogenic microorganisms as a weapon was first revealed in Japan by Ishii Shiro, a bacteriology instructor in a military medical academy, and it found active support among the general staff of the Japanese army.

After the capture of Manchuria and its conversion into headquarters for an attack on the Soviet Union, on its territory in 1932 - 1933 a special bacteriological laboratory was added to a contingent of the Kuomintang army, and it received the code name of "detachment Togo". Ishii Shiro was placed in charge of it. In the following years (1935 - 1936) this laboratory upon the secret mandate of the Emperor of Japan developed the bacteriological weapon in a large plant which even at that time was staffed by as many as 1,300 scientific colleagues and subordinate personnel. In 1940 the staff was appreciably increased and brought up to 3,000 persons. As a cover for the true status of the activities in operation the plant had assumed the name, "Administration of Water Works and Prophylaxis of Divisions of the Kuomintang Army", and later it was called "detachment No. 731". Created as a laboratory, the plant was supposed not only to explore ways and means of carrying out bacteriological warfare but also to manufacture bacteriological weapons in numbers sufficient to supply the combat activities of the Japanese army. In the manufacturing units the agents of plague, cholera, typhoid and paratyphoid fever, and anthrax were cultivated en masse and also fleas, which subsequently were implanted with plague bacteria. The productive capacity was calculated on a yield of 300 kg per month of a bacterial mass of the plague agent concentrated to a sour cream-like consistency, or 500 - 600 kg of the anthrax agent, or 1,000 kg of the cholera vibrio. Units, working on the propagation of fleas, could deliver about 45 kg of live mass per cycle, which lasted 3 - 4 months. In May 1944 the military ministry of Japan demanded an increase in the productive capacity. The yield of fleas in particular was reputed to have increased up to 200 kg per month.

At the start of this program a unit was located in Harbin but later the main part of it was relocated in a special military sector in the district of Pin fan station. The unit was made up of eight sections. The duties of the first (investigative) section were to ex-

explore and study infectious biological agents and to develop methods for their mass cultivation. The second section (experimental) was engaged in the laboratory and field testing of the selected agents, the development of models of bacteriological munitions and sprays, and the propagation of fleas and their infection with the plague agent. The activity of the third section, located in Harbin, was confined to aspects of the water supply and was a mask for the true nature of the unit, but in its experimental workshops shells were manufactured for the bacteriological bombs by the "Ishii" system. The fourth section (production) was reserved for the productive cultivation of the bacteria. In the fifth section (so-called training interval) a corps of officers and noncommissioned men were instructed in the application of the bacteriological weapon under combat conditions. Apart from these the staff of the unit included clinical, supply, and general sections. The unit also laid out a firing ground at the Anta station and a special aviation section.

In the detachment there was a special jail, and its prisoners were used by members of the detachment who, having lost any resemblance to human beings, performed criminal experiments to test the effectiveness of the bacteriological weapon under laboratory and field conditions. It has been documented that not less than 600 persons were exterminated annually by detachment No. 731, and from 1940 until the day of capitulation of the Japanese army the lives of not less than 3,000 persons were forfeited.

In 1940 four related detachments were formed which were located in the line of the main attacks planned by the Japanese operational plan of warfare against the Soviet Union (in the cities of Sunwu and Hailar and at Hailing and Linkoy stations). The branch units were fitted out with industrial equipment for the mass cultivation of pathogenic bacteria and fleas and were combat divisions designed to apply the bacteriological weapon. The plan of operation for combat stipulated the subordination of the branch units directly to the commander of the armies and fronts of the Kuantung forces. The staff of each branch numbered 300 persons.

In 1936, concomitant with the formation of the so-called "Department of Water Supply and Prophylaxis", still another institute of the same kind was created on Manchurian territory and at first received the name (Piroepizootic Division of the Kuantung Army" and later on

"detachment No. 100". The detachment was located in the village of Mokatong, located ten km south of Chan-chun, and like detachment No. 731, it had several branch units. Wakamatsu Tuzihiro (Jiro Wakamatsu?), a major general in the veterinary service, was appointed head of the detachment. The bacteriological weapon designed to infect agricultural animals and plants was developed in the laboratories of the detachment. Possessing a great productive potential, detachment No. 100 cultivated anthrax and glanders bacteria and micro-organisms which would infect grains. The staff of the detachment numbered 600 persons. Bacteriologists, botanists, veterinarians, and chemists worked in its laboratories.

The number of divisions of the Japanese army, which was assigned to the development and application of the bacteriological weapon, was not restricted to the Manchurian detachments of the Kwantung army. There were similar units on the occupied territory of China and even in Japan itself. In 1939 in Canton the detachment, "Nami" 8604, was formed which also had the same job as detachment No. 731. In that very year the same division began to operate in Nanking ("detachment A" 1644). The personnel of the Nanking detachment numbered only a few less than that of detachment No. 731, amounting to 1,500 persons. The creator and commander-in-chief of the detachment was also Ishii Shiro.

The Japanese militarists not only forcefully developed and produced the bacteriological weapon; they even applied it on theaters of military action.

The bacteriological weapon was first used under combat conditions by the Japanese army in 1939 at the time of an attack on the Mongolian Peoples Republic in the region of the Khalkhin-Gol River. While withdrawing to the rear of the Japanese troops, which were devastated by the joint actions of the Soviet and Mongolian divisions, special detachments of the so-called "death divisions" contaminated the water supplies with agents of acute gastro-intestinal diseases.

The bacteriological weapon was also repeatedly used during combat activities in China. In 1940 a special expedition of detachment No. 731, headed by Ishii himself, was sent to Central China. The expedition dispersed fleas infected with plague bacteria from a plane in the district of the town of Ninbo. This operation resulted in an outbreak of plague among the population.

The second expedition into China was organized by

detachment No. 731 in the summer of the following year. The primary task set before the expedition was the impairment of the communications of the Chinese troops, an important junction of which was the town of Chengde. For this purpose infected fleas were again scattered over the town and other population points in the district of Changting Hu Lake and caused an outbreak of plague in the Chinese armies and among the local population.

The third expedition of detachment No. 731 into Central China, organized at the time of the retreat of the Japanese army, considerably surpassed the first two in scope and was planned by the Japanese general staff. The Nanking detachment, "A" 1644, also took an active part in the operation. A specially organized division contaminated the water supply on the territory abandoned by the Japanese army. In some prisoner-of-war camps the prisoners were infected and later released for the purpose of spreading the disease. In abandoned communities contaminated provisions were left. In addition, plague-infected fleas and sprays of bacterial suspensions were dispersed from airplanes. After the end of the operation detachment No. 731 declared at a conference of the directors of the detachment that the application of the bacteriological weapon in Chet'ang district had given impressive results, promoting numerous outbreaks of infectious diseases (26). Akiyama Hiroshi also reported that the detachment practiced the so-called excursions into Manchurian villages for the experimental dissemination of plague-infected fleas.

Preparation of bacteriological warfare was definitely activated after the treacherous attack of Imperial Germany on the Soviet Union, when the controlling clique of imperialistic Japan waited for the opportune moment to invade the Soviet Far East.

This period was associated with the command of the military ministry of Japan concerning a notable step-up in the productive capacity of the detachments and their branches and a remodeling of the producing divisions connected with them, a route for special reconnaissance groups into districts bordering the Soviet Union to find out if the bacteriological weapon could be used, and a number of other undertakings, which positively demonstrated that not by far would the last role in the plans for conducting warfare against the Soviet Union be given to the bacteriological weapon.

As it became known from the testimony of the accused and the witnesses at the trials of the former ser-

vicemen of the Japanese army, who were indicated for the preparation and application of the bacteriological weapon, in accordance with instructions from the general staff a plan of bacteriological attack on our Far Eastern cities - Khabarovsk, Voroshilov, Blagoveshchensk, and Uita - would be developed by the staff of the Kwantun army.

As a part of the "Kan-Toky-A" plan (plan of operation of the Kwantun army for an attack on the Soviet Union, set for 1941) especially prepared courses were organized by detachments No. 371 and 100 for officers and under officers who were assigned to use the bacteriological weapon.

The branch units of detachment No. 731, as this was called in war time, were under the jurisdiction of the commanders of fronts and armies, and in each army there were formed the so-called anti-epizootic units manned by experts from detachment No. 100. The head of detachment No. 731 again was designated General Ishii.

The enumerated undertakings conducted in the period from 1941 - 1945 pointed to the fact that the Japanese army was ready to accomplish a bacteriological attack and was only waiting for a signal. However, these designs were disrupted. The Soviet government, in view of the openly hostile policy of Japan and true to its responsibility to its allies, in order to hasten the end of the Second World War, which was invoking cruel hardships on many millions of people, denounced the neutrality pact and issued an order to its armed forces to destroy the Kwantun army - the shock force of the Japanese aggressor in the Far East. In the briefest period of time this order was fulfilled, and the Japanese armed forces capitulated. The dynamic advance of the Soviet army stunned and demoralized the Japanese troops and did not allow them to use the bacteriological weapon.

Just before the surrender of the Japanese army, in order to conceal the crimes which had been committed, the buildings, equipment, and greater part of the documents of the detachments and their branch units were destroyed, and the personnel were ordered to evacuate to South Korea. Before the departure of the personnel an order was issued which stated that in the event of the capture of members of the detachments the secrets of detachment No. 731 should remain undisclosed without fail. Everyone was ordered to destroy personal identification and other papers proving their participation in the detachment. Although they were far from being all criminals, who developed and applied the bacteriological weapon and conducted felonious

experiments on people, they were successful in getting away from deserved punishment, but some of them, particularly Ishii Shiro, Jiro Watanatsu, and others, fled to Seoul and were taken prisoners by the American army. In spite of the fact that they were the persons who should primarily have had to answer to the verdict of the nation for offenses to humanity, the American powers chose to shield them from deserved punishment so that they could use them in the future in their own interests.

Activities on the creation of the bacteriological weapon had also been carried on for a long time in other countries of the imperialistic camp, particularly in the United States of America.

The first development of the bacteriological weapon in the USA dates, according to official opinion, from 1941. In the fall of 1941 Secretary of War Stimson recommended to the National Academy of Sciences that a special committee be appointed to appraise the possibility and expediency of using pathogenic microorganisms for military purposes. After a detailed study the committee concluded that the bacteriological weapon could be used in combat and urged that speedy measures be adopted. On the report of the Secretary of War it was decided to form a special civilian agency which would direct all activities on bacteriological warfare which were being conducted in the country. Such an organization was organized under the Federal Security Agency and received the name "War Research Service". Merck (38), whose official report we have used for our data reported below, was placed at the head of it. The War Research Service, in the interests of efficiency, economy, and secrecy, was in size a small interdepartmental agency and confined its activities to coordination of the projects, using for this purpose the scientists, working facilities, and experiments of various government and private institutions. The suggestions coming from this organization were vested in the form of orders and directives issued by various military departments, particularly the army medical service and chemical corps.

Apart from the armed forces, the organization had a connection with the Departments of Health, Agriculture, and Interior. The necessary information was furnished by all possible avenues: Army and Navy Intelligence, Strategic Services, and Federal Bureau of Investigation.

The National Academy of Sciences and the Research Council of the USA formed their own special committee, which included many well-known scientists, as an advisory organ for problems of bacteriological warfare.

The War Research Service organized an exchange of information on questions of bacteriological warfare between the USA, England, and Canada. In addition to this, reciprocity of the experts was widely used to coordinate the work.

The most outstanding achievement of the War Research Service, according to Merck, who headed it, was the execution of a program designed to expand the format of the existing knowledge concerning the use of pathogenic agents as a weapon. The most authoritative people in this field of science, particularly Rosebury and his associates, conducted a careful study and selection of all the familiar pathogenic microorganisms in order to determine the advisability of using them for these purposes. The disease-producing microbes, which were chosen, were subjected to a further study in research laboratories of a number of universities and private institutions.

The scope of the expanding investigations soon required a change in the structure of the administration and the routine of conducting the work. The War Research Service requested that the Chemical Warfare Service take over the expansion of the research program and the creation of a powerful pilot-plant for its accomplishment. The site selected for the erection of such a center was Camp Detrick, located not far from Frederick, Maryland. The construction work, begun in April 1943, was completed ahead of schedule. The direct supervision of the activities performed in this center was given over to the Chemical Warfare Service. The War Research Service continued to coordinate the achievements on the bacteriological weapon and defense from it on a nation-wide basis and also to provide Camp Detrick with equipment and a scientific staff.

In 1944 it was decided to pursue the investigations under way by every possible means and especially to solve the problem of the protection of the army, since the intelligence service had reported to the joint chiefs of staff that there were indications that the German command was preparing to use the bacteriological weapon. In view of this a larger part of the responsibility for the fulfillment of the overall program on problems of bacteriological warfare was placed upon the War Department. To direct these divisions of work an advisory organ, the "United States Biological Warfare Committee", was formed in the War Department. The committee included representatives of the Chemical Service, Army and Navy Medical Services, Ammunitions Service, Army Supplies Service, Army



Air Force, a division of the newly formed Department of Special Services of the War Department, Intelligence, and Strategic Services. Merck, who was the director of the War Research Service, was named chairman of the Committee. Because of the transference of authority for the preparation for bacteriological warfare into the hands of the War Department, the War Research Service was liquidated.

The National Academy of Sciences of the USA and the Research and Development Department of the USA, as an advisory organ under the War Department, also organized a special agency, the so-called DEF Committee (39).

Direct supervision of the projects was done by the Special Projects Division, which was a part of the Chemical Warfare Service.

The Army Medical Service was entrusted with the undertakings on the defense of the troops from the bacteriological weapon.

During this period the staffs of the institutes, which were occupied with the development of the bacteriological weapon and the means of defense from it, were appreciably expanded. At the disposal of the Special Projects Division were about 3,900 workers, of whom nearly 2,800 were Army personnel, 1,000 Navy, and about 100 civilian personnel. The number of persons engaged in projects on the bacteriological weapon were far from being confined to the staff members of institutes subordinated to the Chemical Warfare Service. As one of its directors, Brigadier General Creasy (40), indicated, a system of liaison was so extensively used that the development of certain aspects were entrusted to university laboratories and private firms which possessed the facilities for their reliable solution with subsequent remuneration from funds released by the War Department. One can get an idea of the extent of such contacts from the fact that, in Creasy's words (40), "many millions" were expended in payment of them, while the entire cost of the research-developmental work in the field of bacteriological warfare during the Second World War consisted of about 50 million dollars (34).

The variety of agents with which the American experts worked for the purpose of using them as a bacteriological weapon was quite impressive even at that time. Perhaps one can get an idea of this from the incidents of sickness among the personnel of Camp Detrick which appeared as a result of interlaboratory infections. For only three years (1943 - 1946), according to the data of Feiner (41), there were recorded 25 cases of anthrax, 17 of brucellosis, 7 of tularemia, 6 of glanders, and one of

psittacosis. In addition, judging by the projects completed at Camp Detrick and the publications after the end of the war, experiments were also conducted with melioidosis and botulinus bacteria and several species of fungi. It has been said that even the plague agent was subjected to study. Extensive work was performed on the recovery of antihormones intended for the destruction of plants.

In 1944, besides the pilot research-developmental center at Camp Detrick, the Special Projects Division had at its disposal still more centers of the same sort. Specifically under its auspices were the research-developmental centers for field testing - the proving ground at Dugway, located southwest of Salt Lake City (Utah), the one on Horn Island near Pascagoula (Mississippi), and another at the army chemical center in Camp Detrick. At the beginning of 1944 the construction of a building for the manufacture of the bacteriological weapon was completed in Vigo (Indiana) (38).

Besides the War Department and administratively independent of it, a study of aspects of the bacteriological weapon was undertaken as well by the Navy Department of the USA, which set up a number of research-developmental centers. One of them was the Navy Medical Research Center No. 1. which was affiliated with the bacteriology department of the University of California. From an official release of the Navy Department, the contents of which were published in the newspaper, "The New York Times", of 5 January 1946 (42), it is not difficult to grasp that the principal subject of the investigation was the plague bacterium. At the same time work was also being performed on several pathogenic viruses (43). Primary attention was given to the development of methods of making bacterial and viral aerosols. The Navy Department in 1946 issued a statement that the activities of the divisions would continue in the future in accordance with a unified program of the Army and Navy on bacteriological warfare.

The great scope of research-developmental projects helped the United States of America to achieve certain results even by the end of the Second World War in this field. According to Merck, the USA strikingly outdistanced Germany and Japan in this respect.

In spite of the repeated claims of official circles in the USA that the necessity of the expanded operations on problems related to the bacteriological weapon was created only by the threat of its application by Germany and Japan, investigations in this field not only were not

stopped at the conclusion of the Second World War, but they received still more emphasis.

The Chemical Corps centers systematically expanded and increased the volume of the conducted investigations. In 1955 Secretary of the Army, Brucker ordered a new expansion and reorganization of the Corps. In the order it was recommended that the projects be conducted in the realm of creating "new types of bacteriological and chemical weapons to such an extent as only the human brain can visualize (44). According to well-informed sources, the Indian newspaper, "Blitz", reported that in 1956 the National Security Council of the USA had again decided on a further expansion of the Chemical Corps, accelerated production of the bacteriological weapon, and intensification of the research-development programs in this area. An increase in the defense budget for 1957 of approximately three million dollars as compared with 1956, in the opinion of some well-informed observers, was primarily due to the appropriation of large sums for the needs of the Chemical Corps. The number of proving grounds for the testing of the bacteriological weapon was increased. In addition to the proving grounds already at the disposal of the Corps it was planned to assign 400,000 acres for a proving ground in Wendover. New bases for field testings were suggested for construction on the island of Okinawa in the Marshall Islands (73).

Simultaneously a staff of experts was trained in the use of the bacteriological weapon. According to a report in the American newspaper, "Navy Times", such a center was located at Camp Lejeune (North Carolina) - Military School of the Marine Corps. The program of instruction of the officers' division of this school was primarily devoted to a study of the principles of atomic, bacteriological and chemical warfare. Similar schools were also organized in the American occupation zones in Western Germany. In particular, a school of chemical, biological, and radiological warfare, according to the Austrian newspaper, "Osterreichische Volkstimme" (46), was not far from the city of Weyerhormst (Rhine Palatinate).

According to a report in "Newsweek" (47) in the fall of 1954 at Camp Carson and Pike's Peak (Colorado) it was surmised that manoeuvres of the armed forces of the USA included a demonstration of the use of the bacteriological weapon under conditions similar to combat.

A large number of the research-developmental institutions of Western Germany were attracted to the exploitation of the bacteriological weapon. According to the data

of Knobloch (26), activities of this nature were performed at the Nocht Institute in Hamburg, the Hygienic Institute of the I.G. Farben Industries in Barmen, the State Medical Research Laboratory in Laeneburg, the State Research Laboratory in Freiburg, the Biochemical Institute in Tübingen, the Hygienic Institute of the Bayer plant in Leverkusen, and some other centers. Some of the occupational troops of the USA have also worked on the bacteriological weapon in Western Germany: the Army Research Institute in Munich, a number of special laboratories relocated in the area of the so-called "strategic triangle" in the Palatinate (Kaiserslautern-Landstuhl District) and others where German experts were extensively used.

Investigations in the field of the bacteriological have also been broadly carried on in England and Canada. These procedures were started quite a bit sooner in England than in the United States of America. In an official release of the English government (48) it was indicated that as early as 1936 a special agency was created in the Defense Committee in Great Britain whose tasks included a study of problems related to bacteriological warfare. These investigations acquired especially great proportions during the Second World War when the services of a large group of highly qualified specialists were enlisted to work on them, and a huge research-developmental center was established in Porton (49, 50). In 1942 special representatives were appointed in the USA and Canada to establish a liaison on topics having to do with the development of the bacteriological weapon. During the entire war a complete exchange of information was exercised on the achieved results, and there was a reciprocity of specialists. The English government created a special organization - the Division of Microbiological Investigations, which was a part of the Department of Chemical Defense Investigations (51) - to supervise the studies.

The projects were not stopped even after the end of the Second World War and are carried out at present on a large scale. In recent years field testing of the bacteriological weapon has been done repeatedly. Sandis, the Minister of Purchases in England, told of one of them in March 1954 (51, 52). As it appears from his statement, in 1954 regular testings of the bacteriological weapon were advocated in the area of the Bahama Islands, which would have been a continuation of previous testings on the shores of Scotland. One of the factors to be considered in the future testings was a study of the effectiveness of a bacteriological attack on the naval fleet.

Canada also has seven research-developmental cen-

ters which are engaged in projects on making types of bacteriological weapons. One of them is located in Suffield and covers a surface of about 1,500 hectares (74, 80).

The investigations conducted by the USA, England, and Canada are an integration of a unified general program of preparedness of these countries for the application of bacteriological warfare. Special conferences are held for their coordination, and an exchange of specialists is practiced (55). At a later time representatives of the Federation of the German Republic were drawn into participating in these conferences. One such conference including the western German specialists, which took place in Washington in 1954, the newspaper, "Humanité" (56), has noted, referring to the American journal, "Newsweek". It was proposed that the German experts also become familiar with the experimental work at Camp Detrick.

The presented facts assuredly prove that the bacteriological weapon occupies a far from last place in the arsenal of armaments of the imperialistic states.

The Paris agreements specified that the countries - participants in the Western European Military Alliance - would make preparations for chemical and bacteriological warfare and would store up supplies of chemical and bacteriological weapons and would outfit their armies with them.

In Protocol III of the Paris agreements about the formation of the "Western European Alliance", it indicated that members of the block would build reserves of the chemical and bacteriological weapon along with stores of the atomic weapon. These reserves would include, as mentioned in Appendix II of this Protocol, devices and apparatus prepared especially for military use of insect pests or other living and dead organisms or their toxic products.

Protocol IV of the Paris agreements about the formation of the "Western European Alliance" provided for an increase in these reserves in accordance with the number of troops of the state - the member of the block - by a system of mass production of the chemical and bacteriological weapon on the territory of the particular country and by purchasing and receiving from abroad the so-called "external help of military materials" (57).

Preparation of the countries of the imperialistic camp for bacteriological warfare has been carried on in spite of the fact that the application of the bacteriological weapon blatantly breaks the rules and principles of conducting warfare and the universally acknowledged standards of international law.

Prohibition of the bacteriological weapon was es-

established by the Geneva Protocol of 17 June 1925 (the illicit application for warfare of asphyxiating, poisonous, or other similar gases, and bacteriological agents). The members of the Protocol, "considering that the application for warfare of asphyxiating, poisonous, or other similar gases as well as all kinds of similar fluids, substances, and processes are rightly condemned by the universal opinion of the civilized world....., acknowledge this prohibition on bacteriological agents for the conduct of warfare and agree to consider ourselves bound to one another by the conditions of this Declaration" (58).

The Protocol, the effect of which was not limited in time, was submitted for signing, and 40 states ratified or joined in it, including the USSR, Great Britain, Italy, Canada, France, and others. However, some countries, especially the USA, Japan, and others refrained from ratifying it. President Coolidge of the USA presented the Protocol to the Senate for ratification on 12 January 1926, but the Senate vetoed it. The question was definitely decided 21 years later when President Truman on 8 April 1947 withdrew this Protocol from the agenda of the Senate together with eighteen "obsolete" treaties (59).

Refusing to prohibit the bacteriological weapon, which is an agent of mass destruction of the people and which will primarily inflict suffering on the world civilian population, the militaristic circles of the imperialistic states tried in every way to prove that this type of weapon did not differ at all in its character from any other, and therefore the raising of the question about its prohibition not only was not necessary but was even injurious, since their army was deprived of one of the effective kinds of armament. In pages of the reactionary press, especially that of the United States of America, in recent years there have appeared a great number of articles and statements striving to justify in the eyes of public opinion the preparations which are going on for bacteriological warfare. One after another of American generals has come forward, arguing as though the chemical and bacteriological weapons were one of the most "human" agents for conducting warfare. "This may sound paradoxical," declared General Bothchild, "but it can be said that the chemical and bacteriological weapons in the future will be the only hope for comparatively human warfare."

A correspondent of the newspaper, "Christian Science Monitor", in reference to the public address of Major General Stubbs, Chief Chemical Officer, Department of the Army, emphasized that the purpose of this campaign was to "overcome the aversion of the nation toward chemi-

cal and bacteriological warfare" and at the same time to secure new appropriations for its preparation (60).

Major General W. Creasy, (Ret.), former Chief of the Chemical Corps, appeared at a session of the United States Congress in June 1959 with similar assertions (61).

A systematic struggle to prohibit the use of agents of mass destruction, including the bacteriological weapon, has taken place, and the Soviet Union has led and is still leading it. Joining in the Geneva Protocol of 1925, the Soviet government proposed to establish "a very short period for its ratification by all the states" and control of its execution.

In the following years the Soviet Union, at sessions of the Committee on Disarmament of the League of Nations and of the General Assembly of the United Nations, repeatedly has raised the question about taking effective measures to prohibit agents of mass destruction.

In September 1959 during the visit to the USA of N.S. Khrushchev, Chairman of the Soviet Ministry of the USSR, the government of the Soviet Union introduced for the consideration of the United Nations a declaration about universal and complete disarmament with concrete proposals on this question. One of the proposals pertained to questions of the chemical and bacteriological weapon. "The reserves of chemical and bacteriological agents for conducting war which have been accumulated by some states, the declaration stressed, "the poisonous, asphyxiating substances, cultures of deadly bacteria - potential foci of serious epidemic diseases - all of this will be destroyed decisively, without a trace, and forever."

The proposed program of universal and complete disarmament in its third stage provided, among other things, for the inspection of industries for the production, possession, and storing of agents of chemical and bacteriological warfare. All of the existing reserves in the states of the chemical and bacteriological weapon would be confiscated and destroyed under international control (62)

All of progressive world society supported the struggle of the Soviet Union. The International Red Cross which was organized in 1952, directed an appeal to all states to observe the Geneva convention. Even the English medical association joined in this appeal. Before that, in June 1947, the IV International Congress of Microbiologists, convening in Copenhagen, in its resolution directed against the bacteriological weapon, characterized it as "barbarian" and "completely unworthy of civilized society. A very similar resolution was taken by the International Congress of Cytologists.

An important step in the struggle of world progressive society for the prohibition of the bacteriological weapon was the Pugwash (Nova Scotia) International Scientific Commission meeting, which was held at the end of August 1959 at the estate of Cyrus Eaton, a prominent American industrialist and a supporter of world coexistence of countries with different social and political structures. In the work of the conference there participated 26 scientists from the USSR, USA, Canada, England, France, India, Sweden, and Denmark. The staff of the USSR delegation included Academician M.M. Dubinin, Corresponding Member of the Academy of Sciences of the USSR, A.A. Imshenetskiy, and corresponding member of the Academy of Medical Sciences of the USSR, A.A. Smorodintsev. Among the delegates there were high-ranking foreign scientists and specialists: T. Rosebury and Ch. Leak (USA), K. Dolman and S. Watson-Wyatt (Canada), D. Retzlaff and J. Stoker (England), P. Tibeau (France), K. Asanuma (India), Sven Gard (Sweden), and P. Magnus (Denmark).

The scientists gathering in Pugwash joined in one general plea - to use the high achievements of contemporary science and industry for the advantage of humanity and not for the creation of new agents of mass destruction.

At sessions of the conference reports were heard and discussed on the perfidious properties of the chemical and bacteriological weapon.

The message of the head of the Soviet government, N.S. Khrushchev, to members of the conference once more demonstrated to the entire world the firm position of the Soviet Union in the matter of prohibition of all types of weapons of mass destruction - nuclear, chemical, and bacteriological.

The Pugwash conference published an appeal directed against the production and application of the bacteriological and chemical weapon. The opposing stand was that this type of weapon is morehuman than other agents of warfare, but the scientists came forward in favor of the prohibition of the application of the bacteriological and chemical weapon of mass destruction and rejection of secrecy in investigations leading to its creation and adaptation. In a declaration it was stressed that "only the absolute prevention of war will preserve the life of the people and civilization from the chemical, bacteriological, and nuclear weapon" (64, 65, 66).

World public opinion is extremely averse to preparations for bacteriological warfare. The official circles of the USA will be forced to recognize this. In a report



of the Civilian Advisory Committee, approved 6 November 1955 by the Secretary of Defense of the USA, it was noted that chemical, biological, and radiological means of war are put in their proper place in American military plans. At the same time the author of the report acknowledged that the preparation of chemical, bacteriological, and radiological warfare incited everywhere, including the United States, aversion and agitation. "A good part of the activities of the Chemical Corps," it is said in the account, "is considered appalling in character, and therefore it will not encounter any kind of support..... Military circles usually use every means to avoid discussion of the chemical and bacteriological weapon, partly from the risk of international and domestic psychological reactions" (17). The negative position of public opinion is also recognized by the American radio commentator, Fenning, who held a radio interview with General Bullene, chief of the Army Chemical Corps on questions of bacteriological warfare: "A great part of the population of our country reacts negatively to the bacteriological weapon," General Bullene was forced to admit (21).

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The presented facts prove that the bacteriological weapon at present occupies a strong place in the arsenal of some capitalistic states. This arbitrarily dictates an urgent necessity to our medical workers to be well informed on questions of defense from this weapon and to know its basic properties.

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## CHAPTER II

### INFORMATION ON THE BACTERIOLOGICAL WEAPON PROPERTIES AND FEATURES OF THE BACTERIOLOGICAL WEAPON

One of the chief properties of the bacteriological weapon, as many authors will verify, is its potentially high efficacy due primarily to the fact that pathogenic microorganisms and their toxins induce infection, acting on the susceptible host in negligibly small amounts.

Thus, according to Wendt's report (1), Lamanna, a colleague at Camp Detrick, recovered a botulinus toxin, the lethal dose of which for man consisted of approximately 0.15 Y. Still more active was a preparation received at the same center by a member of Abram's group. According to the calculation, although very approximate, one g of this toxin contained about six million lethal doses for man. Live biological agents have a still greater infective potency. Rosebury (2) made the following calculations: white mice succumbed to a subcutaneous injection of a single pair of pneumococci. It is not difficult to compute the weight of these lethal doses if one accepts that the pneumococcus unit, a diplococcus surrounded by a

capsule, has the form of a sphere with a diameter of three  $\mu$ . The volume of such a sphere would amount to  $14 \mu^3$ . Since the density of all living matter is very close to that of water, we can assume that one  $\mu^3$  will weigh approximately one milliard part of a milligram. It follows that one mg of pneumococcal substance would contain 700 million killing doses for the mouse, i.e., 20 times again as many as are contained in one mg of botulinus toxin prepared by Abrams. A still more striking example is with the agent of tularemia. A mouse dies when injected under the skin with one tularemia organism. The size of this microorganism is still smaller than the pneumococcus - 0.5  $\mu$  long and 0.2  $\mu$  in diameter, i.e., its volume consists of 0.02  $\mu^3$ . Conducting similar calculations, it is not difficult to be convinced that this microorganism acts on mice 700 times more potently than the pneumococcus and 15,000 times more strongly than botulinus toxin.

If it were possible to perform similar computations with viral agents, we could probably obtain still more astonishing figures.

But it must always be kept in mind that the figures cited above, obtained by means of rather primitive and arbitrary computations, were constructed on the basis of experiments of parental injections of microorganisms and toxins into the animals, i.e., under laboratory conditions. But in vivo the ratio is different. Rosebury has shown that with the entry of toxins or live agents into the body through inhalation or swallowing the potency of the dose rises strikingly. And these are the most probable routes of entry of the microbes into the body, as it will be seen from further accounts, with the use of microorganisms as the bacteriological weapon. Therefore, considerably more interest is taken in attempts to determine the magnitude of the infecting dose by analyzing interlaboratory infections where the infection of man occurs by the airborne route. Rosebury recalled two cases where attempts were made to determine the dosage causing human infection. One of them pertained to a case of streptococcus tonsillitis of a laboratory worker. Since the length of time she had been exposed and the number of streptococci in the air she breathed were known, it was possible to estimate that she had inhaled about 1, 280 organisms. By means of further calculations it was found that one gram of this culture contained about 700 million airborne infective doses.

The second case of accidental infection, caused by the psittacosis virus, occurred in one of the laboratories at Camp Detrick. A laboratory worker, holding in his hand an ampule containing a suspension of the psittacosis

virus, became aware that it was leaking. A fine spray fell on his palm, and twelve days later he became ill. Later on the probable amount of spilled material was determined experimentally. Further calculations showed that every milliliter of suspension contained not less than 15 million infecting human doses.

The figures obtained in the analysis of these two incidents are considerably closer to actuality than the figures obtained with a single theoretical calculation and pertaining to an animal. However, even here it must be remembered that they refer to a specific, given situation and are not applicable outside of the laboratory. "It is one thing," says Rosebury, "to say that an ounce or quart of any agent contains enough germs to infect so many men and another thing to put this into practice." In reference to this Fox (3) says that "it is rather easy to estimate the lethal dose of a toxin, but it is not so easy to calculate the losses which the bacteriological weapon may bring about." However, one thing is certain: biological agents do have an infecting action in very trifling doses in which none of the known chemical poisons are active.

Specific interest is shown in information on how much of an agent can be introduced into the body by a carrier. Actual experiments have been conducted by Chamberlain, Kissling, and Sikes to decide this question (4). The authors determined the amount of virus of American Eastern equine encephalomyelitis which the mosquito *Aedes aegypti*, infected with this agent, can introduce with a bite. It was established that the amount of the virus entering the body with a mosquito bite consisted of from 100 to 100,000 LD<sub>50</sub> for mice. One mosquito bite was sufficient to produce a fatal course of infection in horses.

Another factor, dependent on the supposedly high infecting potency of the bacteriological weapon, is the ability of some sicknesses to be transmitted from a sick person to a healthy one. Thus, with the use of agents of contagious diseases the number of infected can increase for a long time after the bacteriological attack. Therefore, the theoreticians of bacteriological warfare maintain that a single bacteriological attack on a confined area and with limited agents can create an infectious influence which goes outside of the main infected area and infects significantly more people than those who are subjected to the direct action of the bacteriological weapon. It is not by chance, therefore, that some foreign specialists consider high communicability of diseases one of the most important conditions in an evaluation of the practi-

cability of infectious agents as a weapon (2, 5, 6, 7, 8). The ability of some diseases to be transmitted from a patient to healthy contacts is one of the factors causing the following characteristic feature of the bacteriological weapon noted by foreign experts, namely, the duration of its action (9). Epidemics, which appear as a result of the application of biologically infective agents, can, depending on the circumstances, subsist for a long time. Thus, the period of action of the bacteriological weapon can vary by weeks and months.

On the other hand, as Rosebury has pointed out (5), the duration of the action of the bacteriological weapon is a result of the fact that some microorganisms can maintain their life activities for a considerable time in the external environment. Thus, the agents of typhoid-paratyphoid illnesses are preserved in water for several weeks, and the cholera vibrio for a month. Spore-forming microorganisms can exist under extremely unfavorable conditions for a very long period. In the literature, for example, there is evidence that the anthrax rod keeps alive in the soil for 15 years, and in stagnant soils, which contain many putrescent organic substances, this agent can exist for an indefinite length of time and even accumulate as its reproduction proceeds.

Prolonged action of the bacteriological weapon is possible with the dissemination of biological, infective agents by means of vectors. This is provided, on the one hand, by the fact that the vectors can manifest their activity under favorable conditions when they are strewn on an area. On the other hand, the agents, with which they are infected, are also maintained for a long time in their organism. This concerns agents of a bacterial nature as well as viruses and rickettsiae. Thus, for example, the agent of fever is capable of living in *Ornithodoros nou-bata* ticks for 1,298 days (10), while the virulence of the agent, according to the data of Davis (11), is not diminished. *Ornithodoros parceri* ticks, infected with rickettsiae of Rocky Mountain spotted fever, according to Davis' observations (12), proved capable of keeping alive and transmitted the agent in a bite for a year.

Blanc and Baltazard (13) showed that the agent of melioidosis is maintained in the organism of infected rat fleas for 24 hours, and the fleas are capable of transferring the infection to guinea pigs through a bite.

Chamberlain and Sudia (14) established that *Culex tarsalis* mosquitoes kept alive the agents of eastern and western equine encephalomyelitis and St. Louis encephalitis for practically their entire life, and during the course of



this entire period they are able to transmit the infection to animals. The certainty of transfer of infection has been established by the authors in experiments on rats.

Thus, the longevity of the action of biological agents applied by means of vectors is measured by the life span of the vector. However, for some species of vectors of infectious diseases (ticks) the possibility has been established of transovarian transfer of agents to their progeny. These vectors, finding favorable conditions for their existence, can colonize a locality and cause the formation of a long existing focus of infection.

The essential characteristic of the bacteriological weapon is the presence of a latent period of its action, i.e., the period occurring from the time of application up to the manifestation of the infecting action. This period of time, during which the exposed can maintain a routine and fulfill their duties, will primarily be determined by the length of the incubation period of the illness, the agent of which was used as a biological, infecting agent. It is known that any infectious disease, caused by an attack on the body of live agents as well as of their toxins, is characterized by the necessary presence of the incubation period. The incubation period can be shortened or lengthened, depending on a number of factors (reaction of the body, dose of the infection, etc), but regardless of how many microorganisms or how much toxin attacks the body, the effect is never manifested immediately after exposure or intoxication.

The average length of the incubation period of an illness, agents of which can be applied for a bacteriological attack, fluctuates within broad ranges. Thus, the incubation period of botulism consists of about 12 - 24 hours while that of a fever can be delayed up to 40 days in some cases. In virtue of these factors the after effect of a bacteriological attack is not manifested right away but only after a certain period of time (Table 1).

Another characteristic feature of the bacteriological weapon is the difficulty of its detection. Biological infecting agents do not have any external signs (color, smell). They can be detected only by means of relatively prolonged and intricate laboratory analyses. Some specialists (2, 15) suggest that the bacteriological attack in most cases will be suspected only when the first cases of sickness appear. However, even in this case it will require a great effort to establish whether it is a target of a bacteriological attack or whether an outbreak has arisen naturally, especially where the bacteriological weapon is applied against an epidemiologically unfavorable

locality.

Characterizing the combat qualities of the bacteriological weapon, foreign military specialists note the selectiveness of its action. In contrast to the weapon of

Incubation Period of Some Infectious Diseases of Man and Animals

Table 1

Name of Disease	Incubation Period, in days	Name of Disease	Incubation Period, in days
Anthrax	2 - 3	Tropical fever (dengue)	4 - 5
Brucellosis	5 - 45	Rocky Mountain spotted fever	6 - 7
Tularemia	3 - 7	Western equine encephalomyelitis	4 - 21
Plague	2 - 9	Venezuelan encephalomyelitis	2 - 5
Cholera	1 - 5	Rift Valley fever	5 - 6
Dysentery	1 - 3	Hoof and mouth disease	2 - 10
Glanders	4 - 5	Epidemic gripp	1 - 2
Melioidosis	3 - 14	Occidoidosis	10 - 14
Meningitis	2 - 7	Histoplasmosis	10 - 28
Q fever	14 - 42	Botulinus toxin	18 - 24
Psittacosis	6 - 15		Hour
Tsutsugamushi	6 - 18		
Epidemic typhus	10 - 14		
Yellow fever	3 - 12		

explosive action the bacteriological weapon attacks only living things (16, 17, 8, 18). This characteristic interests the ideologists in the application of this type of agent of mass disease, for they reckon on the seizure of material valuables produced by the peoples, and they therefore rate the bacteriological weapon as "ideal".

Selectivity of action of the bacteriological weapon is also manifested in the fact that the effect of one disease-producing agent is directed only against human beings another only against animals, and a third only against plants. Only a few agents have a complete action.

Many authors note among the properties and features of the bacteriological weapon its enormous psychological effect, which will be very strongly demonstrated when it is applied against an unprepared adversary (7, 8, 19). The appearance of an outbreak of a severe and life-threatening disease, which spreads very rapidly, under certain conditions can evoke a striking psychological reaction. An example of this is the panic in New York in 1947 which was caused by a small outbreak of smallpox (12 cases in all were reported). Many thousands of people stood in line for days in order to get vaccinated.

Some foreign military specialists consider the psychological effect the most important feature of the bacteriological weapon. Franklin Cooper (8), discussing the potentiality of the bacteriological weapon, expressed the opinion that the complete demoralization of the civilian population would probably be the most serious outcome of an unexpected bacteriological attack, and Weissman (7), characterizing the methods and purposes of bacteriological warfare, indicated that bacteria will be used where it is necessary to produce a sustained general panic and disorder.

Discussing the advantage of the bacteriological weapon, foreign specialists note the relative cheapness of its manufacture (2) - most of the microorganisms of a bacterial nature can be cultivated in synthetic nutrient media prepared from comparatively inexpensive and first-quality ingredients. We shall try to clarify some aspects of the problem of mass procurement of biocagents under industrial conditions in the following sections.

#### ROUTES OF DISSEMINATION OF BIOLOGICAL DISEASE-PRODUCING AGENTS

Pathogenic agents can penetrate into the organism of man or animal by different routes: through the mucous

membranes, injured skin, respiratory tract, and also as a result of bites of infected blood-sucking insects and ticks.

Of all the possible routes of transmission of infection the most effective for purposes of bacteriological warfare, in the opinion of most of the foreign specialists is the airborne route. This requires a simple mechanism of infection; the capability of the agents suspended in the air to disperse at decisive intervals and to induce in a brief period the mass affliction of the population (2, 6, 8, 17, 19, 20) and also the complications of defense from airborne infection. It must be remembered that not only agents of airborne infections can be passed on through the air but also microorganisms which normally are not spread by this route (21).

At present because of the voluminous observations, which have been compiled, it has been established that most infectious agents are capable of penetrating and causing sickness in the organism of man or animal by the airborne route. In bacteriology laboratories typhus and other rickettsial diseases quite often appear, although normally these diseases are transmitted, as a rule, only through bites of the vectors. Similar facts have been observed also in the work with viral infections. In view of this a certain interest is taken in an analysis of routes in cases of laboratory infections.

At the present time there are quite impressive data confirming that most interlaboratory infections occur by the airborne route.

As a result of extensive experimental investigations executed at a high technical level, it has been shown that almost all manipulations in microbiological laboratories cause the formation of aerosols which contaminate the air and surrounding environment.

Reitman and Phillips (22) have shown that even the most simple movements with a bacteriological loop give rise to the formation of aerosols. Thus, putting the loop into a test tube or on a plate, placing the inoculum on agar with the loop, the shaking of the loop, and so on form tiny aerosols containing from 0.1 to 8.7 microorganisms (average from ten experiments) into a specific volume of tested air. The aerosol was especially forcefully formed when a hot loop was placed in a dish containing a bouillon culture.

The aerosol was no less intensely formed with laboratory procedures connected with the centrifugation of bacterial cultures.

The results of the authors' experiments proved that even with the simplest manipulations of the bacterio-

logical loop the routine steps in the centrifugation of bacterial suspensions caused the formation of aerosols. The bacterial aerosols were especially intensified when the bacterial mass was resuspended and the cultural contents of the test tube were disturbed.

From the presented findings obtained by experimental means a very definite conclusion can be made that during laboratory procedures with bouillon cultures an aerosol is constantly being formed from the respective microorganisms. The intensity of the aerosol formation depends on the nature of the performed manipulations.

It is very evident that in many laboratory functions not examined by Reitman and Phillips by the experimental means aerosols also may be formed.

But aerosol formation is especially intense in the centrifugation of cultures, and this has also been confirmed by Langmuir (21) in an analysis of incidents of interlaboratory infections.

Thus, in laboratory procedures with bouillon cultures and different bacterial suspensions it is always possible to become infected through the air if protective measures are not applied.

The conditions are still less favorable in this respect in procedures with dessicated cultures. Reitman, Moss, Harstad, Algand, and Stross (23) carried on extensive experimental investigations on aerosol formation with lyophilized cultures. A culture of *Sporotrichum indicum*, dried by the lyophilization method, was used for the experiments.

Aerosol formation was studied with the following manipulations with ampules containing lyophilized cultures:

1. Opening the ampule with a file and then breaking the top off.

2. Pouring the culture from the ampule into the test tube.

3. Dropping the ampule on the laboratory floor.

Air samples were collected with the use of a special apparatus three minutes after each manipulation and were placed on agar.

These experiments showed that even such simple and constantly performed manipulations in the laboratory as the opening of ampules brought about the formation of aerosols. The intensity of the aerosol formation depended on the way the live organisms were dried and concentrated in the ampule.

When the dessicated cultures were seeded in the test tubes containing the bouillon, aerosol formation occurred. The intensity of contaminated air depended on the

drying method and the technique of seeding the dessicated culture. It is not without interest to mention that after the experiment on the seeding of the dessicated culture of *S. indicum* the microorganisms were often isolated from the hands and nose of the laboratory technicians who had performed the work.

Considering that the glass ampules containing the cultures are dropped rather often in laboratories, Reitman et al (23) investigated this aspect of aerosol formation.

The experiments, which were conducted, helped substantially to form very definite conclusions: when an ampule was dropped in the room, an aerosol was formed which could remain in the air for an hour. The intensity of the aerosol formation and the length of its detection in the air depended primarily on the type of drying method used for lyophilization of the cultures.

The presented experimental data very clearly proves that during the procedures with the concentrated fluids and even more with the dessicated cultures, aerosols were constantly being formed in the laboratory which contaminated the air of the room. Therefore, the aerosol route of infection is one of the most probable under laboratory conditions.

It is now well recognized that a large percent of the recorded and studied incidents of interlaboratory infection consists of cases of infection through the air. The most extensive literature is available on airborne illnesses transmitted under natural conditions. Cases of airborne infection have unquestionably been proved in the work with the plague agent. Interlaboratory infections transmitted through the air have repeatedly been described in the work with the agents of tularemia and Q fever (5).

Of special interest are incidents of interlaboratory infections by the airborne method in work with agents the spread of which under normal conditions have not been reported by this route.

It is generally known that brucella infection in man and animals occurs by the alimentary route and also through the mucous membranes of the eyes and the nasopharynx. Isolated incidents pointing to the possibility of the airborne route of infection under normal conditions have still not received recognition since they have not been proved by the necessary factual data. However, cases of interlaboratory infections assuredly affirm the possibility of airborne infection in man with brucella agents.

In the last few years reports have periodically appeared in the literature concerning the infection of laboratory workers through the air with brucella cultures.

Thus, Meyer and Eddie (24), analyzing the case histories of 74 interlaboratory infections with brucella showed that infection primarily took place through the inhalation by the individuals of aerosols of a *Brucella melitensis* culture which formed from the centrifuge in motion. In addition, cases of infection occurred from the dust in pens for sheep suffering from brucellosis.

Langmuir (21) reported on the sickness of a group of scientific workers, who were working in the laboratory with *Br. melitensis*, in a college in Michigan (USA). Altogether 45 persons were affected. In an analysis of the infection route it was established that the infection occurred through the bacterial aerosol formed by the centrifuge in motion.

The minimal infecting dose of the brucella agent in airborne infection of man evidently is very small, which is proved by the infections contracted by individual with single, brief visits to the laboratory (25).

We could go on listing reports of the possible brucella infection by the airborne route. However, even the cited data represent sufficiently ample material (119 interlaboratory infections) supporting the fact that under certain conditions the airborne route of infection by brucella agents suggests an extreme danger.

While the airborne route of brucella infection under natural conditions is not excluded, for some other diseases it has been established only in cases of interlaboratory infection. This primarily applies to the group of communicable infections which under natural conditions are transmitted exclusively through vectors: yellow fever and Venezuelan equine encephalomyelitis.

At the present time the epidemiology of yellow fever has been studied rather exhaustively. It has been established that the only possible route of the transfer of the virus is through the bite of the *Aedes aegypti* mosquito and some other species which are carriers of the disease agent. However, cases of interlaboratory infections have unquestionably confirmed the possible transfer of the virus by the airborne route.

Thus, Berry and Kitchen (26) described seven cases of illness in the laboratory, which were caused by the yellow fever virus, and they compiled a review of 34 interlaboratory infections with fatal outcomes in five cases. Some of the illness were linked to infected mosquitoes but most of the cases arose without any connection with mosquitoes. Some cases of infection were observed in persons who were actively engaged in desiccating the virus. There was one especially interesting case where the person was

in the laboratory only one day and assisted in the work with the infectious material for only a few moments, which shows the exceptionally high infectivity of the virus by the airborne route of infection.

On the basis of an analysis of factual material of interlaboratory infections Rosebury and Kabat (5) and Armagnac (20) quite definitely demonstrated possible infection by the yellow fever virus by the airborne route.

Thus, the experience with interlaboratory infections with yellow fever as an example convincingly shows that a virus illness, transferred under natural conditions only through the bites of mosquito carriers, can also under certain conditions infect people through the air.

There are also similar data about a number of other diseases, which are spread under natural conditions exclusively by the transmissible route, particularly Venezuelan equine encephalomyelitis.

Thus, from the experience with interlaboratory infections and a study of the possibility of becoming infected in the work with infectious material, it can be concluded that human infection through the air occurs not only with illnesses transmitted normally by the airborne route but also with those in which this route of the transfer of the agent is not described or is observed very rarely.

Another possible way of spreading the agent under conditions of bacteriological warfare, in the opinion of foreign specialists, is by the transmissible route. In their opinion the spread of infected carriers has its positive and negative aspects. The possibility of creating a stable focus of infection is considered a positive feature but the negative side is the complication associated with the biology of the vectors and the accumulation in them of the agent (5). Thus, for example, the vectors of yellow fever, Japanese encephalitis, and dengue fever cannot exist in moderate climatic zones and, consequently, cannot be spreaders of infection in these areas. On the other hand, a very complex mechanism of the transfer of the infectious agent is characteristic of some ubiquitous, transmissible infections. This situation is well illustrated by the example of typhus. For the transmission of the typhus virus it is necessary for louse feces to drop onto abraded skin resulting from a bite which itched and was scratched. Still more complicated is the transmission route to man of the bubonic form of plague. As it is known, plague is a typical zoonosis which attacks rodents chiefly. Man is included in the epidemiological chain as



the secondary link. Under natural conditions vectors of plague are various species of fleas. *Xenopsylla cheopsis* - a rat flea which is found in hot, climatic zones - is of the greatest epidemiological significance. The transmission of plague from rodent to man requires a series of definite conditions related to peculiarities in the ecology of rodents and vectors. Man is not a permanent host for rat fleas, and they can only pass over to him and infect him with their bites sporadically. Because of the existing interrelationship between host and vector the plague agent can circulate in nature for a long time (years) as a chain: rodent - flea - rodent -- and man's inclusion in this chain is only a rarity. The complex mechanism of transmission also is the apparent reason why many foreign specialists very mildly speak out in respect to the possible utilization of vectors of transmissible infections as a bacteriological weapon. On this basis Rosebury entirely excludes the bubonic form, which is transmitted by fleas, from potential agents of the bacteriological weapon. Besides these factors the presence of highly effective, completely synthetic insecticides and repellent insect substances have to be considered.

However, in spite of these complexities, from the point of view of application the transmissible route of infection has not been rejected by foreign investigators. It is assumed that under some conditions it can probably prove effective. Many foreign experts hold this viewpoint, evidently mindful of the barbarian experiments of Japanese bacteriologists on the circulation of plague-infected fleas among the inhabitants of the Chinese town of Ningpo(28). As it is seen from the evidence of the Khabarovsk trials the experts in detachment No. 731 attached great importance to sabotage methods of dispersing the bacteriological weapon by using vectors.

The data of recent years have shown that it is possible to develop a breed of insects which have considerable resistance to DDT preparation. This fact also cannot be underestimated in the plan for antibacteriological defense.

Apart from the considered routes of dissemination, the specialists believe that in bacteriological warfare the water route is important, and also the transfer of the agent through contaminated food products (19, 20). However, some authors (2) doubt the possibility of obtaining a significantly damaging effect with infection through the water, considering the dependability of decontamination in modern systems of centralized water works. On this basis the water route is given a minor role, and the possibility

of its use is considered limited (30).

Included in a consideration of possible artificial routes of transmission of infectious diseases there must be remembered still another portal - the entry of microorganisms through wounds. In the opinion of some foreign specialists (3) this route can scarcely play a substantial role in the artificial dispersion of agents. However, in the Japanese detachment No. 731 they considered it possible to spread some resistant microorganisms by such a route, particularly spores of anthrax and gas gangrene (28).

It must be emphasized that most foreign specialists put the airborne route of spreading agents in first place in the premeditated use of them for purposes of warfare. The possible transmission by such a route of even agents of transmissible infections is especially stressed in Rosebury's own works (2, 5) and in that of Armagnac (20). In their opinions the removal of vectors from the chain of usual transfer of infection might allow, first of all, the dispersion route to be greatly simplified and, secondly, the use of agents of transmissible diseases even in those climatic zones where the respective vectors cannot exist.

In respect to this it is necessary to analyze in great detail the problems related to the formation and action of bacterial aerosols.

## BACTERIAL AEROSOLS

Dispersing systems with a gaseous atmosphere and a solid or liquid dispersing phase are termed aerosols.

It is customary to term aerosols with liquid particles as clouds, and aero-dispersing systems with solid particles - depending on the mechanism of their formation - as dusts or vapors.

According to the mechanism of formation of the aerosols they can be classified as dispersing and condensing.

The first is formed with the dispersion (dusting, pulverizing) of solid and liquid particles, and the second as a result of the condensation of supersaturated vapors or gaseous reactions which produce nonvolatile products.

The term, bacterial aerosol, refers to aero-dispersing systems, the liquid or dry particles of which bear microorganisms.

In the opinion of foreign specialists either bacterial clouds, where the liquid culture is sprayed, can be made for the application of biologically infected agents or dry preparations - bacterial dusts - can be used. According to the mechanism of formation the artificial bac-

terial aerosols naturally will always be dispersable. On the basis of the material existing in the foreign literature it is possible to assert that the pathogenic activity of the bacterial aerosol in the final analysis depends on the kind of microorganisms applied, their concentration in the air, and also on the dispersing ability of the system. The relation of the potency of the pathogenic action of the bacterial aerosol to the qualities of the agent subjected to spraying does not demand special explanation. In addition, the pathogenic potency of the aerosol is found in direct relationship to its concentration, i.e., the number of microorganisms in a unit volume of air.

How then is the concentration of microorganisms determined in a bacterial cloud apart from their number in the original dispersing products? There is voluminous literature on this question from abroad, an analysis of which helps us to make some generalizations. First of all the bacterial aerosol represents a system, the behaviour of which is determined by the laws of physics as well as by biological principles. In connection with this, an answer to the posed question can be obtained only after a thorough acquaintance with the physical and biological properties of the aerosol.

The behavior of the aerosol as a physical system depends on a number of factors and particularly on the size of its particles and the kinetics of the gaseous phase.

The size of the particles in aerosols fluctuates within very broad ranges - from 1 mm to 0.01  $\mu$ . The shift from the lower range to the upper is accompanied not only by quantitative changes of almost all the properties of the aerosol but also by variations in the nature of the principles supporting these changes. Thus, the rate of settling of the particles of the aerosol is clearly related to the evolution of its liquid or solid phase. This

can be illustrated by data borrowed from Frank's table (Table 2).

Table 2						
Diameter of particles in microns ①						
Rate of settling ② in cm/sec						
Character of seed- ③ ing	④ Settled at uniform rate				⑤ Moving like gaseous mol- ecules	

① Диаметр частиц в микронах	1000	100	10	1	0,1	0,01	0,001
② Скорость оседания в см/сек	400	30	0,3	0,0035	0,000035	0	0
③ Характер оседа- ния	④ Оседают с постоянной скоростью				⑤ Двигутся подоб- но газовым мо- лекулам		

Theoretically the lower limit of the size of the particles of the bacterial aerosols apparently within the range of 1 - 5  $\mu$ . Further reduction in their size is limited by the size of the bacterial cell as well as by the possible disintegration of the suspensions and dusts. The upper boundary of the size of the particles is determined by the stability of the system. In systems with stable gaseous atmosphere the particles with a radius of several hundred microns settle very quickly. There is a high sedimentation rate also in particles with a diameter of 10 - 100  $\mu$  (0.3 - 30 cm/sec), but it must be remembered that these indices pertain to systems with a stable atmosphere. Turbulence in the atmosphere noticeably slows down the sedimentation rate of particles suspended in the air. Sedimentation even of such huge particles as those 50  $\mu$  in size takes place in the open atmosphere not due to the force of gravity but because they are brought to earth like molecules of a gaseous cloud. The latter mix and are brought to the ground due to turbulent diffusion. Next to the ground the layer of air is more or less immovable, and the particles permeate through it virtually by settling. Not all the particles settle by any means, which are adjacent to the ground, and some are again snatched up by rising air currents and move on (Johnston, Winch and Smith (31)).

From what has been said one has to conclude that

the behavior of the aerosol cloud is determined not only by its dispersing ability but also by the kinetics of the gaseous phase.

Evaluating the influence of these factors on the efficacy of bacterial aerosols formed in the application of the bacteriological weapon, Fothergill (32), a consultant at Camp Detrick, emphasizes the importance of meteorological conditions. He indicates that the aerosol can exhibit an optimal damaging effect only with certain micro-meteorological conditions. The principal meteorological factors, which determine the direction of movement of the aerosol cloud and its diffusion, are the force and direction of the wind and the extent of vertical resistance of the air. The greater the force of the wind, the faster the aerosol is dispersed and the concentration of the agent is reduced.

The vertical resistance of the air is characterized by the temperature gradient, i.e., the difference between the temperature of the air measured at a height of 20 and 150 cm from the surface of the ground. Three classes of vertical resistance around the ground layer of air are distinguished - inversion, convection, and isothermal.

Inversion (gradient is less than 0 degrees) is observed at night time with a cloudless sky and a mild wind which does not exceed 4 m per second. With inversion there are no rising air currents, and therefore the aerosol cloud will disperse slowly and hang over low places and forest masses for a long time.

With convection (gradient is greater than 0 degrees) there is a rising air current which favors rapid diffusion of the aerosol cloud. This state is observed in clear weather and with little cloudiness.

With an isothermal condition the temperature of the upper and lower layers of air around the ground is almost the same (gradient is within ranges of -0.2 to +0.2 degrees), as a result of which there are no vertical air currents, and the aerosol cloud will disseminate slowly. Inversion is observed for short intervals in the summer time, longer with cloudiness, and for a long time in the winter with dense cloudiness.

Fothergill (32) considers that the application of the bacteriological weapon, designed to contaminate surface layers of air, is expedient only with meteorological conditions of the isothermic or inversion type. With a positive gradient the cloud will rise. Disintegration of the bacterial aerosol as a result of the settling of the particles does not cause the biological agents to lose their pathogenic potency. Settled microorganisms contami-

pollute the soil, water works, combat equipment, clothing, and other things found in the path of movement of the aerosol bacterial cloud. This can be an additional source of infection for people who are present on the contaminated territory, either as a result of contact with the contaminated objects or with the consumption of contaminated food and water, and also as a result of the inhalation of the restored aerosol (7) which comes about through the repeated rising of the settled microorganisms into the air with dust.

An infection zone can be formed in this area as a consequence of the settling of the microbes on the ground, and its effectiveness will be determined by the resistance of the biological agent, and again by the meteorological conditions, and by the character of the locality (relief, soil, vegetation). With the formation of a stable focus of infection the use of spore forms of microorganisms, particularly anthrax, is more probable (2, 29, 33).

The fate of the bacterial aerosol depends on a number of other factors which show an influence on the survival of the microorganisms. Fothergill (32) primarily specifies solar radiation, which quickly inactivates microorganisms suspended in the air, relative humidity, and temperature. The biological peculiarities of the microorganism dust and the methods of pulverization are very important too. Thus, spore forms of microorganisms are very well preserved in an aerosol, and vegetative ones die quickly. In experiments conducted by Rosebury (34) and his colleagues it was shown that while the percent of restoration of *B. prodigiosum* from the aerosol did not exceed 7.5, this value consisted of 73.4 % for the spore-forming microorganism, *Bac. globigii*.

Processes causing the decay of microorganisms develop even as the aerosol is being developed. To what extent these take place depends, as has been shown by Rosebury, on the construction of the sprayer. In the first place there is the injurious effect on the microbial cells of the force of air with discharge from the sprayer.

In the cloud formers, the construction feature of which is the presence of a deflecting shield, the damaging factor is the impact of the particles against the shield.

With the use of a generator type of aerosol cloud former the destructive effect on the microorganisms can also be produced by an increased concentration of salts in the suspension in the vaporization process.

The processes, which destroy the microorganisms and which are connected with the mixing of fluids in the reservoir of the percussion sprayer, have been carefully

studied by the English authors at Horton (35, 36, 37). Similar to the preceding two, this factor is characteristic only of cloud formers and is not a feature of direct sprayers where the liquid in the tank is in a static state during its operation.

Thus, the presented data reveal that the decay of microorganisms sprayed into the air begins from the moment of generation of the aerosol and depends on the construction of the sprayer as well as on the routine of the operation.

The decay of microorganisms goes on at a later time also.

The detailed investigations conducted by Dunklin and Luck (38) have helped to establish that the death of bacteria sprayed from a suspension depends to some degree on two independently proceeding processes. One always takes place more rapidly, and as a result of it a large number of microorganisms die during the first 5 - 20 minutes of the life of the aerosol. The second decay process of the microorganisms proceeds more slowly and stretches out for many hours.

Later Perry and Marie (39) confirmed the accuracy of Dunklin and Luck's conclusions.

The intensity of the bacterial decay in the aerosol, due to the rapidly proceeding process, depends on a whole series of factors, particularly on the magnitude of the relative humidity, the temperature of the air, the sustaining environment, the type of microorganism, and the size of the particles.

Data on the influence of relative humidity on the survival of microorganisms sprayed into the air has very controversial for a long time. Williamson and Gotaas (40) experimenting with *B. prodigiosus*, *S. coli*, and staphylococci concluded that the most favorable conditions for the preservation of their viability were created with low indices of relative humidity. This point of view found its support also in the works of Edwards, Alford, and Laidlaw (41) Loosli, Lemon, Robertson, and Appel (42), who showed that the grippe virus, dispersed into moist air, died significantly sooner than in dry air. De Gne established that the percent of destroyed *S. pullorum*, dispersed from an aqueous suspension, steadily rose with an increase in the relative humidity. Conversely, Ellis and Tapscott (44) have maintained that hemolytic streptococci die quickly in a dry atmosphere and are preserved very well in a moist one.

An explanation of the contradiction in these conclusions probably is found in the fact that the authors,

studying the importance of relative humidity, did not consider the influence of other factors which activated the interrelationship.

A careful control of humidity and temperature conditions along with standardized methods of aerosol formation, a sample intake and determination in it of the concentration of viable organisms helped Dunklin and Fack (3) to explain many of the differences. In the conduction of experiments with pneumococci sprayed from a bouillon suspension with different indices of relative humidity (from 3 to 80%) and different temperatures (14, 22, and 33 degrees), they established an extremely interesting fact - the most intensive death of microorganisms occurred in a narrow range of relative humidity (around 50%). With lower and higher indices the pneumococci survived for a considerably longer time. The same, but somewhat less expressed regularity, was also demonstrated in experiments with staphylococci and streptococci.

An explanation of the mechanism of accelerated destruction of microorganisms in the first minutes after spraying into the air with average indices of relative humidity, in the opinion of the author, must be sought in the processes arising in the droplets of fluid falling into an atmosphere which unsaturated by water vapors. Evaporation of water from such droplets at first leads to an increase in the concentration of substances dissolved in the liquid surrounding the microorganisms; some of them can be toxic for bacterial cells. The bacterial cell itself at this time also loses the water present in it and with intense drying becomes resistant to different kinds of unfavorable chemical and physical actions, and therefore the destructive effect of substances contained in the droplet of fluid surrounding the bacterial cell, the concentration of which increases as a result of evaporation, becomes significantly lower.

The amount of water lost by the cell is clearly related to the relative humidity of the air. With very low indices of humidity dehydration proceeds so rapidly that the microorganisms become resistant to unfavorable effects, not having time to be exposed to the destructive influence of the rising concentration of salt dissolved in the fluid drops.

The excellent preservation of microorganisms in the aerosol with high indices of relative humidity has to be explained by the fact that both in the cell itself as well as in the drop of fluid surrounding it there remains a sufficient amount of water, and the concentration of salts dissolved in it does not rise again or rises negligibly.



Very unfavorable conditions for the cells are created with intermediate indices of relative humidity, when the cell remains sensitive to the toxic reaction of dissolved substances which become more concentrated as a result of evaporation.

The temperature of the air also shows a great influence on the resistance of microorganisms in the aerosol containing fluid particles. The present theory is that with different temperatures the relative humidity shows a varying influence on survival.

Brown (45, 46, 47) demonstrated substantial differences in the survival rate of bacteria in an aerosol at 0 degrees and 10 degrees. If with a temperature of 0 degrees the lowest decay rate for all species of the bacteria under consideration (*B. coli*, *Achromobacter*, *Pseudomonas*) was registered at a relative humidity of 70%, then at 10 degrees this value corresponded to the maximal decay of *Achromobacter*. With a temperature of 10 degrees and a lowering of the relative humidity below 60% a sharp increase in the decay rate of *B. coli* and *Pseudomonas* was noted.

Kethley, Fincher, and Cown (48), studying the influence of temperature in a range of from -40 to -32 degrees on the survival of *B. prodigiosum* in an aerosol, also established that with various temperatures the relative humidity exhibited a varying influence on the survival of bacteria in the aerosol. In their opinion, in hot weather (with the exception of very dry air) the possibility of the dissemination of airborne infection is minimal due to the slight resistance of microorganisms in the aerosol under these conditions. With more moderate temperature regime favorable conditions were formed for the microorganisms with high indices of relative humidity. With low temperatures the bacteria in the aerosol were resistant to a broad range of relative humidity indices.

The fate of microorganisms sprayed from the suspended state also depends on the dispersing capacity of the aerosol. If the decay of bacteria sprayed into the air is explained by a process associated with dehydration, then the death of microorganisms, with other conditions equal, should proceed more intensively in large dispersing aerosols as compared with small systems, since in drops of a smaller size, containing a smaller amount of substances acting harmfully on the cell, the microorganisms in the dehydration process are subjected to the action of their lower concentrations. In the work of Dunklin and Fack (38) already cited above (38), this premise has been confirmed by experiments with the spraying of pneumococci from a

bouillon suspension, in which it was established that the more intense drying out took place in aerosols with large drops.

The presented data reveal that the first process, causing the destruction of microorganisms in the aerosol and happening in the first minutes of its activity, is connected with changes in the osmotic and chemical activity of the cells and is the result of drying. The intensity of the dying out of the sprayed microorganisms depends on those states where dehydration occurs. Dunklin and Luck consider that this same principle is at the base of the lyophilization process which is used for the preparation of dried, viable cultures of microorganisms.

An analogous point of view on the nature of the decay process of microorganisms in aerosols is upheld also by Rosebury (34), who assumes that the evaporation of water from the drops of spray is the reason for the death of the vegetative microorganisms. This supports his theory that the selection of the suspending medium favors an increased survival of bacteria in the aerosol. The experiments conducted by him and his associates substantiated the accuracy of this deduction. It has been shown that with the spraying of *B. prodigiosum* suspended in distilled water the restoration index averaged 0.9%, while in the spraying of a culture suspended in a gelatin solution, it rose to 14.1%. It was also established that in a certain range of the concentration of the solution of this preparation an influence was shown on survival.

Similar data have also been recovered with other test agents. It has been demonstrated that the best suspending media for the agents of glanders and melioidosis are glycerin and bouillon, for the agent of brucellosis - a mixture of dextrin and one of the products of protein breakdown (2).

These experiments are the basis for recommending that a different type of stabilizing supplement be added to the culture which is intended for spraying (Rosebury - 34).

The second process must be dwelt on briefly which causes the decay of microorganisms in the aerosol and which proceeds for a longer time than the first and independent of it. This process compares qualitatively with the decay of microorganisms in dried preparations. In the opinion of Ferry and Marle (39) it is related to slow oxidation. Its intensity depends on the species of microorganism, the suspending medium, and the relative humidity (49, 50, 51, 52).

## SOME ASPECTS OF INFECTION BY BACTERIAL AEROSOLS

With its occurrence in a bacterial cloud infection can take place through various portals of entry. These may be exposed parts of the body with damaged skin (wounds), gastro-intestinal tract, conjunctiva, and respiratory organs. Inasmuch as infection through the skin and the digestive tract is not common for airborne infection alone, some features of infection will be considered in this section only by the inhalation route, which is specific and the most important just for airborne infection.

Certainly the most important in the application of the bacteriological agent is inhalation infection which arises as a result of the penetration of the agent into the body through the respiratory organs.

According to the data established at the present time (34, 53), the character and features of the action of the bacterial aerosol on the body in general (not considering the state of the microorganism) is determined by:

- the biological and physical-chemical nature of the aerosol;

- the amount of biologically active particles retained in the body, i.e., the dose of infection;

- the initial distribution of these particles in the body;

- the future fate of the retained particles (removal, re-distribution, resorption, etc).

The number of microorganisms, which enter the body from the bacterial aerosol through the respiratory tract, is determined by three basic factors: the concentration of the aerosol, exposure (length of inhalation), and the extent of pulmonary ventilation. The inhaled dose of the infecting agent is calculated by the formula:

$$D = ctv_t$$

where D = dose of infecting agent; c = concentration of biologically active (live) microorganisms; t = exposure;  $v_t$  = extent of pulmonary ventilation.

The indicated method of calculation is not sufficiently accurate and even under conditions of experimental work in an aerosol chamber gives errors reaching 55 - 60%.

The difficulty in determining the inhaled dose is due to the common intricacies of calculating a pure concentration of aerosol and the extensive individual fluctuations in the respiratory volume of the subjects. In

In addition, the correct estimation of retainment of the inhaled aerosol in the body is of essential importance, and this will be discussed below.

The dose of the infecting agent is decidedly important in the development of a disease. As the investigations of Rosebury (34) have demonstrated, the inhalation infecting doses and lethal doses vary with different diseases in the first place, and secondly, they sometimes overshadow corresponding doses in subcutaneous and other modes of infection (Table 3).

Table 3

Comparative data on infectivity of biological agents with different routes of infection (Rosebury)

Agent	Brucel- losis	Meliodosis	Glanders	Tularemia		Psittacosis (doses in intracereb- ral units ID-50)	
(Number of organisms in ID-50)							
Method of infection	guinea pig	mouse	hamster	ham- ster	mouse	mouse	mouse
Inhalation	130	100	70	160	70	15 1860	20
Intraperitoneal	6		15	20	1	25	124
Subcutaneous	6	7	15	20	1	25000	1850
Intracerebral	-	-	-	-	-	1	1
Intravenous	-	-	-	-	-	46	-
Intracutaneous	10	15	-	-	1	-	-
Cutaneous (scarification)	-	-	-	-	159	-	-

**Aerosol Retention in the Body.** It is known that with the inhalation of an aerosol, including the bacterial one, only part of the inhaled particles is retained in the body since the rest escape into the external environment with breathing. Consequently, the degree of entrapment of the inhaled aerosol will to a significant extent influence the dose of the infecting agent which the body receives.

The settling and entrapment of aerosol particles in the respiratory tract are caused by three fundamental factors: force of gravity, strong inertia, and Brownian movement. With this the force of gravity determines the sedimentation (settling) process; inertia causes the impingement of rectilinear (with the air) moving particles against the surface of the respiratory tract where there are curves, narrowing, or roughness; Brownian movement of very tiny particles also brings about their attachment to mucous membranes (53).

The primary characteristic determining the magnitude of gravity, inertia, and Brownian movement of the aerosol particles is their mass, i.e., the size of these particles. Therefore, the dispersion capacity of the aerosol is the most important factor determining the extent of its entrapment.

Important, but not decisive, points in this process also are: the concentration of the inhaled aerosol, the depth and frequency of respiration (extent of pulmonary ventilation, the type of respiration (nose or mouth), and the state of the respiratory organ.

The electrical charge of the particles, their hygroscopic nature, and other physical-chemical properties show a certain influence on the extent of entrapment (53).

There are extensive, diverse data in the literature on the aspect of the degree of entrapment of dry and liquid aerosols in the body. However, in summary, considering first of all recent investigations with the use of the most modern methods and apparatus, it is possible to generalize them in the following manner (53, 54).

Величина частиц в микронах	10 и более	5-10	3,2	0,8-1,6	0,4	0,22
Процент задержки	100	80-100	61-80	19-48	17-33	19-36

- 1) Size of particles in microns
- 2) 10 and more
- 3) percent of entrapment

<sup>1</sup>A cross-section and other anatomical-physiological characteristics of the respiratory tract are discussed with application to man.

From the presented data it follows that the largest particles are chiefly influenced by the force of gravity and inertia, as a result of which they settle principally in the upper respiratory tract. With a decrease in dispersion the influence of gravity and inertia abates, and on the whole these particles are held back and penetrate into the deeper parts of the lungs. Particles  $0.3 - 0.4 \mu$  in size have little gravity and inertia with practically unexpressed Brownian movement; as a result, the percent of retention of this fraction is the smallest, and those  $0.3 - 0.4 \mu$  in size do the least settling. With even smaller particles the influence of the force of gravity and inertia is also decreased, but Brownian movement is significantly heightened, which again causes greater retention.

The concentration of the inhaled aerosol also influences the extent of retention in the body (55). The opinion originally existed that an increase in the concentration caused less retention of the particles. This idea, however, was later disproved, and the fact was established of a direct relationship between these sizes: with increase of concentration of the inhaled aerosol the extent of particle retention rose in the body.

The amount of pulmonary ventilation also shows an influence on the extent of retention, but an estimation of it is controversial. Thus, Owens (56) established that with the inhalation of an aerosol with a concentration of 5,560 particles in  $1 \text{ cm}^3$  (dispersion capacity  $0.85 - 1.7 \mu$ ) a shift from a quiet type of respiration to deep caused an increase of 26 - 28 to 79% in the retention. These data were not later confirmed by Brown (55) and other authors who, on the other hand, concluded that the percent of retention was inversely proportional to the extent of pulmonary ventilation.

The type of respiration - nose or mouth - substantially influences the extent of retention of the inhaled aerosol.

It has been established by numerous investigations (55, 57, 58) that the percent of aerosol retention with a nasal type of respiration notably surpasses that with the mouth type. The reason for this phenomenon, in the opinion of the author, is found in the air vortexes and the loss of speed of the particles with their passing from narrow passages of the respiratory tract to wider ones.

The difference in the degree of retention with various types of respiration is manifested the most for particles  $1 - 5 \mu$  in size, since this fraction alone is held back mainly in the nose. In addition, it is known that the type of respiration does not show any essential influ-

ence on the retention of the coarsest dispersed particles and the small dispersed ones: a coarse aerosol is well retained with mouth as well as with nose breathing, while the most highly dispersed particles settle poorly with respiration both through the nose and the mouth.

The electrical charge of the particles causes a higher percent of retention in the body. Possibly a charge favors an immediate settling of the particles in the respiratory tract and thus insures a more stable state of the aerosol and a longer time for the preservation of its original concentration.

Other properties of the particles, like their specific gravity, hygroscopic nature, ability to form conglomerates and flakes also influence the extent of aerosol retention in the body, but their role has not been sufficiently studied.

Thus, the primary factors determining the inhalation dose of the infecting agent are the concentration of the aerosol, exposure of its inhalation, and extent of pulmonary ventilation. The main factor influencing the extent of retention of the aerosol particles in the respiratory organs is the dispersion nature of these particles.

**Distribution of Retained Particles in the Respiratory Organs.** The dose of the infecting agent undoubtedly plays a decisive role in the development of the infectious disease. Along with this a material influence on the pathogenesis of the disease is shown by the infection routes which often are determined by the sites of the primary application of the agent. With airborne infection, when the inhalation route of infection takes place, the microorganisms can be implanted in the body along the entire course of the respiratory tract beginning with the nasal orifice and ending with the alveoli. If there is added to this the fact that resistance to infection in various parts of the respiratory tract is not the same, the role of the primary distribution of inhaled bacterial particles becomes still more understandable (32, 59, 60, 61, 62).

It has been established that the basic factor determining not only the extent of retention of inhaled particles but also their primary distribution in the respiratory system is their dispersion nature (32, 54, 57, 58, 64, 65).

A summary of results of numerous investigations on the distribution in the respiratory organs of aerosol particles, depending on their size, allows us to conclude that particles more than 50  $\mu$  in size are retained chiefly in the upper respiratory tract (nose, mouth, nasopharynx),

particles of 30 - 50  $\mu$  can penetrate into the trachea; 10 - 30  $\mu$  do not impinge deeper than the bronchi; 3 - 10  $\mu$  not beyond the bronchi and, finally, particles 1 - 3  $\mu$  in size and smaller can reach the alveoli where they completely settle (54, 57, 58, 63, etc).

Along with these generalized data many authors have been successful in demonstrating even coarser particles of 5 - 13  $\mu$  (66, 67, 68) and even 80  $\mu$  (69) in the alveoli.

If one estimates that the diameter of the portal of entry into the alveoli consists of 70 - 100  $\mu$ , the infiltration into them of particles of relatively large size has to be considered as quite plausible.

No less important are the data on the weight distribution of semi-dispersed aerosols in the respiratory tract.

Abramson (63), studying the implantation into the lungs of experimental animals of crystalline penicillin powder with an original dispersion capacity of 0.65 to 58  $\mu$  established that the total amount of inhaled preparation was distributed according to weight in the respiratory organs in the following manner: alveoli - 0.65%; alveolar passages - 5.91%; borderline bronchi - 45.15%; trachea - 45.15%. Thus, it was graphically shown that with a semi-dispersed consistency of the aerosol the basic mass (according to weight !) impinged and was retained in the trachea and bronchi.

And so, on the basis of literary data, it is possible to conclude that with the initial distribution of the aerosol in the respiratory organs the relatively large particles were retained in the upper respiratory tract and did not penetrate into lung tissues; only particles not exceeding 3 - 5  $\mu$  primarily impinged and were retained in the alveoli. Along with this, with the inhalation of the semi-dispersed aerosol the greatest percent of particles according to weight were detected in the upper respiratory tract, trachea, and bronchi, while in the alveoli a small amount of inhaled substance was detected.

**Further Fate of Particles of Bacterial Aerosol.** If all of the basic physical rules pertaining to biologically inert particles are applied in general in the processes of penetration, retention, and initial distribution in the body of particles of the bacterial aerosol, then the further fate of bacterial particles is determined chiefly by biological principles and concepts of the interaction of micro- and macroorganisms. Thus, in particular, in an appraisal of the biological action of the bacterial aerosol on the body often the quantitative factors, which characterize, for



example, the total amount of retained particles, yield to a second course before such biological properties of the given microorganism as virulence and aggressiveness, which frequently determine the nature of the infectious process and the fate of the organism introduced into the body. Nevertheless, some general rules, well-known in respect to inert particles, are common to particles of bacterial aerosols; a presentation of them will help us to advance clearly the further behaviour of these particles in the body.

The fate of the particles of the bacterial aerosol which penetrates into the respiratory organ and settles there is not the same: one escapes from the body into the external environment, another penetrates into the gastro-intestinal tract and, lastly, a third undergoes resorption.

Relatively coarse, dispersed particles escape mainly from the body and settle principally in the upper respiratory tract. Due to the activity of ciliated epithelium these particles move toward an outside opening and escape along with the secretions from the nose and nasopharynx in coughing, sneezing, and blowing the nose. Thus, although the large particles are well retained in the respiratory tract, nevertheless they are rapidly and most completely removed from them (20, 21).

Small and minute particles, a certain percentage of which penetrate into the deep sections of the respiratory tract, are also retained in the alveoli, escaping to a significantly lesser degree, but this process still does take place. At first phagocytosis of these bacterial particles by the leukocytes and histocytes occurs and then they are removed from the body through the bronchi by a current of air (72, 73).

Penetration into the Gastro-Intestinal Tract occurs with ingested particles of the aerosol which have settled in the nose, nasopharynx (nasal type of respiration), and mouth (mouth type of respiration). With the inhalation of coarsely dispersed aerosols, when the percent of retention in the upper respiratory tract is high, the number of particles impinging against the gastro-intestinal tract can be especially high, markedly surpassing the number impinging directly onto the lungs (57, 58, 71, 74).

Inasmuch as the genesis of disease is not specific for airborne infections by alimentary routes, the further behavior of organisms in the gastro-intestinal tract will not be considered.

Resorption of particles of the bacterial aerosol can occur from all surfaces of the respiratory tract. Organisms penetrate through the barrier of mucous membranes

of the respiratory tract and alveolar epithelium like biologically active particles suspended in mucous secretions.

Numerous experiments with inert and poisonous (strychnine, curare, etc) substances have shown that the absorbing capacity of the respiratory apparatus is extremely high; its absorption often exceeds the rate of resorption with other mucous membranes, particularly of the gastro-intestinal tract. Such an intensive resorption is explained by the properties of the mucous membranes of the respiratory tract and alveolar epithelium as well as by their enormous surfaces (75, 76).

These data have also been verified by recent investigations of American authors; they have specifically established that for botulinus toxin a minimal lethal dose with airborne infection is several thousand times less than with alimentary infection (32).

It is also known that through the respiratory tract there occurs a more active resorption of substances which are poorly absorbed through the digestive organs (75).

However, the intensity of absorption for various parts of the respiratory tract is the same. Heubner (75) established that resorption proceeds more energetically through mucous membranes covered by ciliated epithelium (respiratory tract) while it takes place less well through mucous membranes covered with many layers of flat epithelium (mouth, throat, esophagus, opening to the larynx). In general, it can be concluded that absorption proceeds from all surfaces of the respiratory tract, but its intensity gradually increases from the nasal orifice to the deep parts of the organs, reaching a maximum in the alveoli.

The mechanism of penetration of the organisms through the barrier of mucous membranes and alveolar epithelium has not been adequately studied. It is necessary, however, to keep in mind that in many infectious diseases the penetration of microorganisms occurs on a background of more or less manifested local inflammatory changes in the respiratory tract or lungs, which, as a rule, combine with the general action of the agent on the body. Undoubtedly the destruction of epithelium local vascular disturbances, and innervation disturbances, like a weakening of defense mechanisms - particularly phagocytosis - aid in the penetration of organisms. The properties of the organism play a large role in the process: its morphology, virulence, aggressiveness, etc.

Microorganisms penetrate through the mucous membranes or alveolar epithelium directly or indirectly, being previously subjected to phagocytosis (77, 78). As a result, passing through the mucous membranes directly or

through special intracellular gaps, the microorganisms impinge on the sinusoides layer of the connective tissue. Later on they either enter the blood capillaries of the lungs directly and are carried along them throughout the entire body, or else they enter the lymphatic vessels and from there the regional lymphatic glands, and then they penetrate the thoracic duct and the venous blood (79).

Depending on the nature of the pathogenesis of the illness and of the mode of infection by the bacterial aerosol (dispersing capacity and primary distribution of particles), the harmful organisms will proceed in the body along the course of the whole respiratory tract beginning with the nasal orifice and ending with the alveoli.

The best conditions with this for surmounting the natural physiological barriers are created right in the alveoli and deep parts of the organs (32).

Experimental observations and clinical experience have shown that, depending on the site of settling of the pathogenic agent, various forms of sickness can develop with characteristic pathogenesis. Thus, with the introduction of the tularensis organism to the tonsil area there develops a tonsillar-bubonic form of tularemia, while the impingement of the agent against the deep parts of the lungs induces a pulmonary form of illness. Infection with the plague organism through Waldeyer's ring can cause bacteremia and the development of a secondary pulmonary form of plague; along with this, the deep inhalation of the agent produces plague as a primary disease. Examples of such a kind are very numerous (80, 81, 82).

A summary of data, devoted to the question of airborne infection and published in the foreign literature, permits us to make the following conclusion.

An inhalation dose of the infecting agent, being derived from the concentration, exposure, and volume of pulmonary ventilation, proves to be the most important factor determining the onset and course of infection. The extent of retention of aerosol particles in the body with breathing considerably influences the magnitude of the inhalation dose, and this depends first of all on the dispersing capacity of the aerosol; large-sized particles are retained in the respiratory organs to quite a greater percent than small ones. In addition, the degree of retention is directly proportional to the concentration of the aerosol and the electric charge of its particles; the nasal type of respiration increases the percent of retention.

The primary distribution of particles in the respiratory organs plays an important role in the pathogenesis

of airborne infection, the nature of which is also determined by the fractional dispersing makeup of the aerosol. Larger particles settle in the upper respiratory tract while the small and minute ones penetrate and are retained in the deep parts of the bronchial tree and in the alveoli. The greatest retention of particles with this in respect to weight occurs in the upper respiratory tract.

Later on the particles retained in the respiratory tract may be eliminated from the body (particularly the larger ones), be ingested in the gastro-intestinal tract (also the coarser fraction), or be subjected to resorption (absorption).

The respiratory system has especially favorable conditions for absorption. The implantation of organisms through the epithelium of the respiratory tract and alveoli can occur along their entire course but the most intensive resorption takes place in deep parts of the respiratory tract, especially in the alveoli.

It must be considered that the invasion of organisms into the body very often occurs on a background of more or less expressed local pathological changes in the respiratory organs, which break the natural barrier functions of the mucosa and promote the penetration of organisms into the body at any point.

Depending on the site of invasion of the agent, the pathogenesis and clinical picture of the disease can essentially change, which allows us to conclude that the primary application of the infecting agent (distribution of aerosol particles) shows an influence on the course of the infectious process.

Serious consideration is given abroad to the study of the nature of infection by bacterial aerosols, and especially in the USA at the present time this problem is the center of attention of American experts on the bacteriological weapon.

#### PROBABLE OBJECTS AND PURPOSES OF A BACTERIOLOGICAL ATTACK

Acquaintance with American and English literature devoted to the application of the bacteriological weapon allows us to presume that different authors do not have a single point of view.

Some authors assume that the bacteriological weapon can be applied for the infliction of a blow only along the deep rear of the enemy; others consider that it is universal and can be used for the contamination of objects both

in the rear as well as on the field of battle.

In American official directives (9) it is pointed out that the most probable objects of a bacteriological attack will be mobilization and embarkation centers, large concentrations of troops in front and rear lines, industrial centers, and also the agricultural economy and large expanses of agricultural plants.

Armagnac (20) considers that the bacteriological weapon is a strategic one and can be used for disruption of the work of rear objects: military structures and other commercial enterprises, transportation systems and electrical stations. Analogous opinions are held also by Wiesman (7) and Fothergill (32).

Rosebury (5), analyzing this aspect, also puts the strategic goal in first place. In his opinion the most expedient use of the bacteriological weapon is to resolve the following problems:

- attack of insular, sea and air basins which are not considered to be occupied by armies of the side using the bacteriological weapon;

- submission of surrounded urban fortresses;

- disorganization of industrial districts at the rear of the enemy;

- infection of army camps and centers of troop training;

- infection of abandoned territory upon strategic withdrawal;

- infection of animals on cattle ranches and of agricultural crops.

Application of the bacteriological weapon during military action when troops of the belligerent side are in close contact, Rosebury (5) considers hardly probable but still possible.

The authors, assuming that the bacteriological weapon cannot be used for a resolution of tactical problems, base their point of view on the danger from the retroactive effect. The term, retroactivity, is based on the possible infection by the bacteriological weapon of troops of the side using this weapon. The retroactive effect can arise either with the application of the agent of contact diseases, an outbreak of which will spread even among the armies of the side using it, or with the contamination of terrains by stable biological agents with the subsequent advancement of troops along this territory (5).

Discussing the problem of the retroactive effect, Sartori (83) cites an example from the time of the First World War when the Germans made an attempt to infect cattle in France with hoof and mouth disease. This attempt was

successful - an epizooty arose, but it spread to Germany, too, where even more cattle died than in France.

The possible application of biologically contaminated agents for an infliction of a blow at troops, who are actively engaged in military operations, is limited to the presence in the bacteriological weapon of a latent period of action, during which time the exposed can maintain their routine. Under conditions of a rapidly changing setup this fact to a known degree will lessen the value of the military effectiveness of the bacteriological weapon (2).

The authors, indicating the possibility of using the bacteriological weapon to resolve not only strategic but also tactical problems, assume that the danger of the retroactive effect can be significantly lessened if a series of precautionary measures are taken by troops of the side applying this weapon. Among them are included: choice of those biologically infecting agents which would neither cause the emergence of an epidemic nor permanent contamination of a terrain, immunization of personnel and their provision with agents of individual defense, etc (5).

#### TECHNICAL AGENTS OF APPLICATION

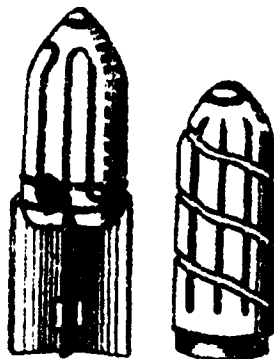
Many foreign specialists in works devoted to questions of bacteriological warfare are presenting their views on the possibility of using various technical agents for the application of the bacteriological weapon. Most of them consider that there can be used for this purpose specially constructed containers, pouring and spraying aviation devices, aviabombs, mines, artillery shells, etc (2, 5, 8, 17, 34).

In the opinion of foreign specialists the bacteriological bombs and shells should be small in size with relatively small-sized explosive shells and easily destroyed casings. In accordance with American views such small ammunition will not be applied by means of single explosions but in a series similar to the incendiary bombs thrown down in packets (9).

Light metal, earthenware, glass, or plastic can serve as material for bacteriological shells (9). Thus, for example, earthenware casings for the body of the bacteriological bombs were developed in the Japanese detachment No. 731. This bomb was prepared from a special kind of clay by means of drying it in a plaster form and firing it in a special kiln. The length of the bomb was from 70 to 80 cm, the diameter was 20 cm. The body of the bomb was filled with small flasks of plague-infested fleas, and

a small amount of explosive substance was walled up in special channels located in the walls of the body. In addition, a remote control tube was placed in a special opening underneath the bomb. Such bombs were meant to be, in the opinion of their creators, thrown from an airplane and exploded over the ground. Very little explosive substance was required to explode the ceramic bomb. This insured the preservation of the fleas with the explosion (28) (Figure 1).

Fig. 1.  
Ceramic Casing for  
the Ishii Bomb



In the opinion of foreign specialists the bacterial agents can be applied also in splinter shells, mines, grenades, and other ammunition. In the opinion of Rosebury there can be used with the greatest effect in these ammunitions stable forms of microbes, which are capable of forming spores - for example, agents of anthrax, tetanus, and gas infection.

Aerial bombs can be thrown from a plane directly onto the ground, or they can be dropped in special parachutes. Besides the bomb, shell, mine, and container various spraying devices or pouring tanks mounted on planes can be used to distribute the bacteria or infected vectors (9, 86).

Foreign specialists do not exclude the possible application of bacterial agents by saboteurs. More than that, American military specialists recognize this as an ideal weapon for various kinds of sabotage (87).

As it appeared from the evidence of the Khabarovsk trials, the Japanese at the time of the war in China developed and tested on Chinese servicemen sprayers of plague infected fleas in the form of fountain pens and canes. This type of sprayer was reserved for saboteurs. In addition, the saboteurs could attempt to contaminate water basins with agents of infectious diseases. For this purpose, as Rosebury has indicated, it is possible to use thin-walled glass ampules. The ampules upon falling into the water either broke under the effect of their own weight or under the influence of gas forming with contact

of the certain element with the water. Another modification of the container for agents or toxins conveyed by water - for example, botulinus toxin - could be a paper filter made from a carton or porous paper and impregnated with substances soluble in water (2).

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### CHAPTER III

#### SOME PROBLEMS IN THE MASS PROCUREMENT OF BIOLOGICAL AGENTS.

According to the current views of foreign specialists the demands of bacteriological warfare can be satisfied only by the mass procurement of microbial material on an industrial scale (2). In view of industry's success in the production of antibiotics it has been demonstrated that it is indeed possible to accomplish a similar feat in cultivating pathogenic bacteriological agents in huge quantities. In the United States of America in the state of Indiana an experimental plant is in operation which was constructed for these purposes and was created as long ago as the beginning of 1944 (1). In the opinion of American scientists (2, 3, and others) the principles of mass obtainment of organisms under modern industrial conditions is general for all microorganisms, for pathogenic as well as for those producing antibiotics, some vitamins, and other products of current fermentative production. The system existing in business for the production of antibiotics and in laboratories developing vaccines, as Rosebury indicates, can be shifted over to the manufacture of

the bacteriological weapon without any special changes. The cultivation technique of the microorganisms is also similar.

In contemporary microbiological industries the cultivation of microbial cultures for diverse purposes is accomplished in a cultivation apparatus (or fermentors, reactors) in liquid nutrient media. The capacity of the modern cultivators is measured in tens of thousands of liters (4).

The culture of pathogenic microorganism grown in the apparatus, in accordance with the opinion of Rosebury, may be the starting point for outfitting special containers and the later application for the affliction of people or animals. However, considering the negligible durability of liquid cultures with storage, the American specialists, as it has already been noted, consider it worthwhile to preserve the bacterial cultures in the desiccated state and to use them in that same form (5). Therefore, the technology of procuring bacteriological agents under industrial conditions also provides for the preparation of dried microbial cultures.

The available foreign literature on the problem of the mass cultivation of microorganisms allows us to conclude that the principal plan of industrial procurement of organisms consists of the following basic steps:

1. Preparation of nutrient media for cultivation.
2. Cultivation in a fermentation apparatus.
3. Preparation of fluid and dried biological cultures.

Let us turn now to a consideration of individual aspects of the technology of industrial procurement of microorganisms.

In reference to the common opinion about a single principle of the industrial procurement of microorganisms, in the present chapter a general theory will be stated concerning the mass cultivation of pathogenic as well as of nonpathogenic microorganisms in the light of the current foreign literature.

#### PREPARATION OF NUTRIENT MEDIA

One of the fundamental problems determining the possibility of mass procurement of microbial material is the industrial requirement for a sufficient amount of the necessary nutrient media. The need of pathogenic organisms for nutrition is very striking, and the mass preparation of biological agents inescapably entails a large stock of raw materials.

For growth and development the microbial cell should receive from the nutrient medium all of the elements contained in it in the right amounts. In connection with this it is relevant to dwell briefly on the chief questions related to the nutrition of microbial cells.

The growth and development of microorganisms are associated first of all with the synthesis of protein, the most important element of the cell. Pathogenic organisms, being typical heterotrophs, are capable of assimilating nitrogen only in the form of amino compounds from high molecular nitrogenous substances which are products of protein breakdown. These include peptones, polypeptones, and amino acids. Protein substances are easily assimilated by the bacteria after preliminary splitting into amino acids. The process of the splitting of protein molecules occurs under the influence of proteolytic enzymes liberated by the microbial cells. However, the proteolytic activity of bacteria is extremely varied. Thus, for example, it is sharply manifested in the agent of an anaerobic infection and very weakly expressed in the plague agent. In connection with this the assimilability by the cells of various products of protein decomposition depends on the intensity of the splitting of the protein substances entering into the composition of the nutrient medium.

In addition, for the growth and development of microbial cells certain organic compounds are necessary which cannot be synthesized by the organisms from the surrounding environment and have to be supplied to the cells in a usable form, i.e., they have to be in the nutrient medium. These include some amino acids and also purine and pyrimidine rings. These compounds enter into the composition of the cytoplasm. As Werkman and Wilson have shown (6), "all the cells, whatever their origin, contain protein which with hydrolysis gives a mixture of the same 18 amino acids in different proportions".

Innate to each species of microorganism is its quantitative requirement of the indicated amino acids in the nutrient medium.

There exist organisms which require 2 - 3 amino acids, but for some of them the number of essential amino acids is about 18. There has not been found a need for more than 18 amino acids in a single microorganism (6).

A study of the amino acid requirement in *B. anthrax* has shown that these microorganisms grow well on a mixture of amino acids, among which are leucine, isoleucine, and valine (7). For the growth of the plague organism, according to the data of Rao (8), the necessary amino acids are phenylalanine, cystine, and proline. Although it is not

essential, the amino acid, glycocoll, stimulates growth. However, this view is not shared by some American authors, who maintain that only a medium containing all of the amino acids which make up casein secures the growth of all strains of the plague organism (9).

Of the mineral ions needed for the full development of organisms  $K^+$ ,  $Mg^{++}$ ,  $Mn^{++}$ , and  $Fe^{++}$  are necessary, and some require  $Ca^{++}$ ,  $Zn^{++}$ , and  $Cu^{++}$  (10, 11). In addition, the growing microorganisms have to have sulphur and phosphorus (12). The requirement in the nutrient media of the indicated ions is dictated not only by the fact that they determine the extent of osmotic pressure and the ionic state of the medium but also by the important role of some ions in various functional processes of the organisms.

However, even in the presence of all these substances the growth of bacteria is impossible if the corresponding physical-chemical conditions favorable for growth are not formed.

An important factor supporting the growth of cultures is the reaction of the medium (pH). For each species of organism there exist certain ranges of pH, and only in these ranges does the normal growth of the microbial population occur. Besides these conditions the corresponding temperature and partial oxygen pressure are necessary and the presence of growth factors - vitamins, belonging chiefly to the B group (13, 14, 15, 16, 17).

The basic nutrient media, with which until recent times the work has been performed in microbiological laboratories, are those prepared in meat bouillon with the addition of various peptones obtained by the enzymatic hydrolysis of protein products. Peptones are a mixture of albumose, polypeptides, and amino acids, and they are unstable and fluctuate depending on the raw material and the method of hydrolysis. Waste products from the meat industry (blood, fibrin, parts of the stomach of cattle, and other protein products) are used as the raw material for the recovery of peptones. Protein hydrolysis is accomplished with the help of proteolytic enzymes: pepsin, trypsin, and pancreatin. It is not difficult to see how enormous amounts of these costly substances could be consumed to meet the needs of microbiological production on a large scale. In addition, the process of enzymatic hydrolysis itself is sufficiently complicated and comprehensive in the sense of the technology and standardization of the obtained products. Therefore, the analytical brain has for a long time worked on the simplification and lowered cost of production, first of all along the direction of substitution of costly raw materials by cheaper and more

available sources.

In recent years in vaccine production the preparation of nutrient media has expanded still further by the method of acid (and also in some instances by alkaline) hydrolysis of protein products. The recovered protein hydrolysates are aqueous solutions of various amino acids which are formed with the extensive decomposition of the original protein products as a result of their treatment with sulfur or an acid salt. The method of acid hydrolysis has greatly simplified and made cheaper the preparation of nutrient media, since instead of deficient enzymes (pepsin, trypsin, pancreatin) accessible acids have come to be used. Various wastes of biological plants, canning factories, defense and similar plants may serve as the raw material for acid hydrolysis. By the process of acid hydrolysis it is possible to work out and achieve in this way the specified decomposition of protein products in the nutrient medium which is to be used for the cultivation of any microorganism. Considerable success in the development of nutrient media with the use of the method of acid hydrolysis of protein has been accomplished by many authors. As the starting products for hydrolysis there are selected the most varied substances of a protein nature.

Favorable results in the cultivation of bacteria have been obtained by Gladstone and Filodes (18), Lorentz (19), and Wolf (20) with the use of a medium based on hydrolysates of casein and cottage cheese.

McManon (21) prepared acid hydrolysates from fish products, and Fisher (22) from fishbone meal.

Some authors have used the wastes of the meat industry for acid hydrolysis, for example, blood (23), gelatin (24), etc. The authors indicate that the disadvantage of acid hydrolysates is the lack in them of mineral salts, humous substances, and some colloids, which inhibits the growth of microorganisms.

Nevertheless, the given method, in spite of some disadvantages, is broadly used at present in vaccine production. Purification of the protein hydrolysates from wastes and harmful impurities is not an insoluble problem. For this purpose activated charcoal and various ionic exchange resins are now being applied.

By means of the adsorption of ions, which carry positive or negative charges (cations and anions) and in a like manner by the corresponding resins (cationites and anionites) acid hydrolysates are successfully obtained with the necessary concentration of mineral ions. Khimuro (25) used ionic-exchange resins for the purification of an



acid hydrolysate of casein from an excess amount of chlorine ions and humous substances. As a result of purification the hydrolysate became colorless and transparent. The chlorine content in it dropped from 8.2 to 0.15 mg/ml.

It is possible to extract the necessary amino acids from the hydrolysates by a similar method. Removal of the amino acid mixture in the protein hydrolysates with the help of ion-exchange resins can be used in industry for the procurement of purified amino acids and peptides, which are needed for the preparation of high quality and effective nutrient media for microorganisms, as the investigations of Partridge and Brimley (26), Consden, Gordon, and Martin (27), Paulson and Deatherage (28) and other authors have shown.

Lessening the cost of the nutrient media for microorganisms has also branched into the use of plant proteins in their preparation. Some plant proteins, according to their content of indispensable amino acids - arginine, histidine, lysine - are similar to proteins of animal origin. Bean plants (soy, peanut, pea) are especially rich in first-class proteins. In contrast to animal products the plant products contain somewhat more carbohydrates and less protein and fat.

The first attempt at cultivating pathogenic bacteria on nutrient media was proved by Tsenkov as early as 1877. Later Pawlowsky (29), Sander (30) conducted extensive investigations on the cultivation of tubercle bacilli on vegetative media, especially potato medium. On the basis of comparative experiments Sander decided on the advantage of vegetative media over meat. Hook and Fabian held the same opinion (31). According to their data nutrient media, prepared from vegetative wastes, best favors the growth of bacteria.

Attempts are also described in the literature to use asparagus for the cultivation of fungi to be used for antibiotics (32) and also soy (33) and bean-like lupine plants (34, 35) to prepare nutrient media for bacteria.

Repeated attempts have also been undertaken to prepare nutrient media by the method of acid hydrolysis of protein products of vegetative origin.

There must be mentioned still another raw material which is used to prepare nutrient media, namely, yeasts. Yeasts are a valuable nutrient substrate, very similar to meat. They have a high content of nutrient substances and vitamins.

For the preparation of nutrient media yeasts are first autolyzed with the help of the protease contained in them (pepsinase, tryptase, eriptase). Yeast media are

used in epidemiological practice in the preparation of vaccines (36).

In recent years the attention of investigators has turned more and more to the possibility of using corn extracts for the preparation of nutrient media. As Kennedy, Speck, and Aurand (37) have reported, corn extract contains a mixture of amino acids and a substance (Corn-Steep factor) which stimulates the growth of bacterial cells. In addition, it promotes the development of penicillium by the fungi producers (38), and in relation to this corn extract media has been used in the penicillin industry for a long time (4, 40, etc). The possibility of using other herbaceous plants for nutrient media has been shown by Moyer and Coghill (41).

Thus, by numerous works on the use of various protein substances as basic components of nutrient media there has been established a real possibility of replacing products deficient in high-quality meat substances with more available and cheaper ones. It is even more important to stress this because, in the opinion of foreign specialists (2), under war time conditions foods, especially meats, become "strategic material", and their use for other needs, apart from the feeding of the population, is scarcely possible.

The requirement of microorganisms for nutrient substances has been extensively enough studied by now. It has been established that for the growth and development of many organisms, including the pathogenic ones as well, the presence of protein in the medium is not needed; it is enough to have only some amino acids and mineral salts. The most important point with this is the proportion and concentration of amino acids in the medium (42). This fact serves as the springboard for the development of a new trend in microbiology - the preparation of synthetic nutrient media of a certain composition in relation to the aminogram for the cultivation of microorganisms. In many modern microbiological laboratories they now prefer to work with synthetic media. The possibility of changing the makeup of the medium in the necessary direction by leaving out or adding different amino acids and other ingredients in exact quantitative proportions allows the investigators to comprehensively study some aspects of the physiology of the microorganisms.

The basic principles presented in this section on the preparation of synthetic nutrient media pertain exclusively to microorganisms of a bacterial nature.

The cultivation of viruses and rickettsiae differs strikingly in concept from that of bacteria and occupies

a special place.

In contrast to bacteria, viruses and rickettsiae do not multiply when placed on artificial medium. They can be cultivated only in the presence of living cells. At present there are two fundamental methods with different modifications for the cultivation of viruses and rickettsiae: infection of the chick embryo and cultivation on animal tissue.

Most of the pathogenic viruses and rickettsiae known now are cultivated with success in the chick embryo, its membranes, the chorionic membranes, or the extra-embryonic fluid. An impressive amount of virus material is obtained with this. Therefore, the given method more and more finds extensive application in investigative work and in the preparation of diagnostic antigens and vaccines.

Embryos of other domestic birds (duck, turkey) are also used for the cultivation of viruses and rickettsiae besides the chick embryos, but the latter are the most practicable because of the shorter incubation period as compared with other embryos, a higher susceptibility to most viruses and rickettsiae, and also their low cost (43).



Figure 2. Mass preparation of chick embryos

In our survey there is no necessity to analyze in detail all the techniques concerning the infection of embryos with viruses. These techniques have been exposed at length in a number of textbooks and manuals on virology. The use of the given method for the marketing of vaccines and diagnostic preparations in modern industrial enterprises makes it possible to acquire viruses and rickettsiae

in mass amounts. And this, as Rosebury has indicated, can if necessary, satisfy the requirement completely in the production of viruses and rickettsiae for the purposes of warfare.

In recent years in viral laboratories the use of the method of viral and rickettsial cultivation in growing cultures of various animal tissues has been put into practice on an increasingly extensive scale (44). Many kinds of tissue can be used for this purpose, either of the embryos or adult animals, most often of birds and mammals. The chief ingredients of such cultures are living cells obtained from the corresponding animal and physiological solution.

There are several formulas for salt solutions for tissue culture. The most generally used are Tyrode's and Ringer's solutions.

The minced tissue from different embryos ("embryonic extracts") is usually added to the medium as a nutrient substrate.

The Maitlands, and later Lee and Rivers (43) established that the vaccinia virus of smallpox multiplied favorably on a medium composed of pieces of tissue in Tyrode's solution. The method of tissue cultures developed by Lee and Rivers is the simplest of all the previously used ones and is widely used at the present time in scientific-investigative laboratories for the cultivation of viruses and rickettsiae.

#### SUBMERGED CULTIVATION OF ORGANISMS IN INDUSTRIAL APPARATUS

The interest in the problem of cultivating microorganisms on a large scale under industrial conditions has become especially noted abroad in the last 15 - 20 years. The development and organization of antibiotic production is one of the chief reasons for this.

The basis of the current productive acquirement of microbial material is cultivation accomplished in a fermentation apparatus (cultivator) by the method of submerged cultivation. At the present time examples of fermentators of various capacities intended for the cultivation of non-pathogenic and pathogenic microorganisms have been worked out and described in the literature (40, 45, 46, 47, etc).

All foreign authors have arrived at the single opinion that, irregardless of construction, the apparatus should answer a definite requirement, providing the submerged cultivation of organisms with aeration of the med-

ium. The general requirements of an apparatus intended for the cultivation of nonpathogenic and pathogenic organisms have been formulated in the work of Malmgren and Heden (48) and consist chiefly of the following: the apparatus should be airtight in order to exclude possible contamination by extraneous microflora from the surrounding environment and to prevent the escape of the cultures into the external environment for the safety of the personnel. Therefore, in the planning of cultivators special attention is given to the construction of various valves, joints, and gaskets. Thread joints, packings, and so on are used only in extreme cases and are replaced by welded joints with flat seams at the elbow to facilitate exhausting. All the supply lines and fixtures are made with this in mind so that they will be suitable for steam sterilization under pressure (3).

Along with the need for an airtight condition the authors indicate that the cultivator apparatus should provide at the same time excellent aeration of the nutrient medium, i.e., its saturation with oxygen. A sufficient inflow of oxygen to the growing culture is one of the most important prerequisites for the method of submerged cultivation and for the large volumes of liquid nutrient media.

The fact has long been known of the growth stimulation of microorganisms with agitation and mixing (or shaking) of the growing culture, so that the medium becomes more intensely saturated with oxygen (49, 50, 51, etc).

The most complete aeration can be accomplished by means of bubbling or blowing air (oxygen) through the medium, and this also depends on the plan of construction of modern industrial cultivators or fermentors.

Using both principles of aeration in the cultivation of brucella in a laboratory type of cultivator, Brown (52) recovered a yield of 50 - 80 milliard of viable organisms in one ml of culture. With this, depending on the amount of air blown through, cultivation was reduced from 20 to 2 1/2 hours. These and other similar experiments convince us that the culture yield occurs in a direct ratio to the extent of aeration (53, 54, 55).

Chain and associates (46) have shown that effective aeration is one of the primary requirements in the technology of submerged cultivation of microorganisms.

In modern industrial cultivators the medium is aerated by means of blowing through air with the help of bubblers of various construction and also by mixing the medium with the use of special devices - stirrers. This insures the breaking up of air bubbles into smaller ones and the additional mixing of the air bubbles with all the

liquid particles. As a result, the oxygen is intensively diffused, and the length of the phase during which the air and liquid are in contact is prolonged. Dunning and Truesdale (56) have demonstrated that the coefficient of oxygen diffusion in the medium is increased with an accelerated rate of mixing. There are indications that the preliminary humidifying of the air going into the apparatus greatly influences the effectiveness of aeration and that this enables better diffusion in the liquid and the most complete absorption by the microorganisms.

However, intensive mixing and aeration favor the formation of a large quantity of foam in the cultivator. Foster (4) showed that the formation of froth evidently is associated with the synthesis of fatty acids, which in an alkaline reaction resemble soap and make a stable foam in the protein. The foam, which has formed, exhibits a negative reaction on the course of the cultivation, significantly decreases the optimal capacity of cultivation, markedly reduces aeration, clogs up the pipe-lines and gaskets, and can disrupt the technological process (46, 52, 58, etc). Therefore, special fixtures to combat foam formation are considered in the construction of the apparatus.

An important condition of submerged cultivation in the apparatus, according to the comments of Malmgren and Heden (48), is an assurance of a stable state for the cultivation process. It is important that the nutrient medium and the culture are not subjected to any unmanageable influences which might affect the growth of the bacteria. To guarantee uniform operational conditions in the apparatus it is outfitted with fixtures that record and regulate the individual parts of the technological process. The fixtures allow the temperature in the apparatus always to be kept at the necessary level, also record the amount of oxygen and carbon dioxide in the gaseous mixture which is used for aeration, and constantly maintain the flow rate of the gaseous mixture. The hydrogen ion concentration of the culture must be watched continually, and it must be adjusted by the addition of the respective reagents. It is also desirable to have a device which will automatically register the turbidity of the culture as an indirect indicator of growth (48).

The construction features of the cultivation apparatus, according to Perret (59), apart from the enumerated requirements, may be contingent on the pathogenicity of the cultivated organism, its means of nutrition, and the growth rate.

We will present a short description of a cultivator of standard construction which is similar to the one

referred to in the work of Chain (46).

The cultivator is cylindrical and is made of rust-proof steel. To insure effective sterilization of the cultivator itself as well as of the medium put into it, the apparatus is furnished with a steam jacket (3, 4).

The inside surface of the cultivator is smooth with no rough places. All of the interior details are assembled and fastened to the cultivator cover which is freely attached to the body with the help of bolts or "autoclave" locks (47) securing airtightness at the joining surfaces. In modern apparatus of large capacity they do not make a cover; the apparatus is manufactured in the form of a cylinder with spherical bottoms welded to it. The cylinder stands vertically, and all the mountings are arranged on the upper base; running through it are the agitator shafts and pipes which supply and discharge the air for aeration, the tubes for the introduction of seeded material and necessary supplements during fermentation, the tube for the sample intake, and some recording devices.

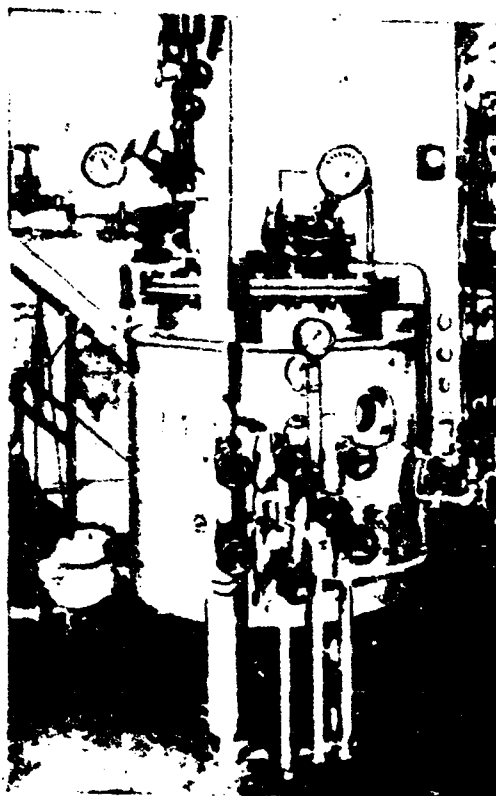


Figure 3. Standard type of 300 liter cultivator for non-pathogenic microorganisms.

In the very upper part of the apparatus there is located an inspection window and a trap door enclosed by a hermetic lid which is used for the cleaning of the apparatus. The agitator shaft goes through the center of the upper foundation. The outside of the shaft is supported by ball bearings, and they are connected by pulleys or reducers to a motor. The stirring appliance can be of various constructions: propeller, turbine, or in the form of a cone. It is recommended that all of these details, as well as the agitator shaft, be made of rust-proof steel (3).

The delivery of air to the fermentor for aeration is accomplished through dispersers of various construction. In the literature there are described ring, square, spiral and other agitators with openings differing according to their size and number (from one to several hundred) for the discharge of air (46). Air, escaping under pressure from the agitator through the tiny openings is further broken up into particles by the action of the agitator (Figure 6).

A high degree of air dispersion can be obtained with the use of an ejector type of mixer or by special adapters which provide a greater velocity of air flow (47).

In the fermentor of Garibaldi and Feeney (45) the air bubbles coming out of the disperser were additionally broken up by means of a four-blade propeller located directly over the ring-shaped disperser. For better mixing



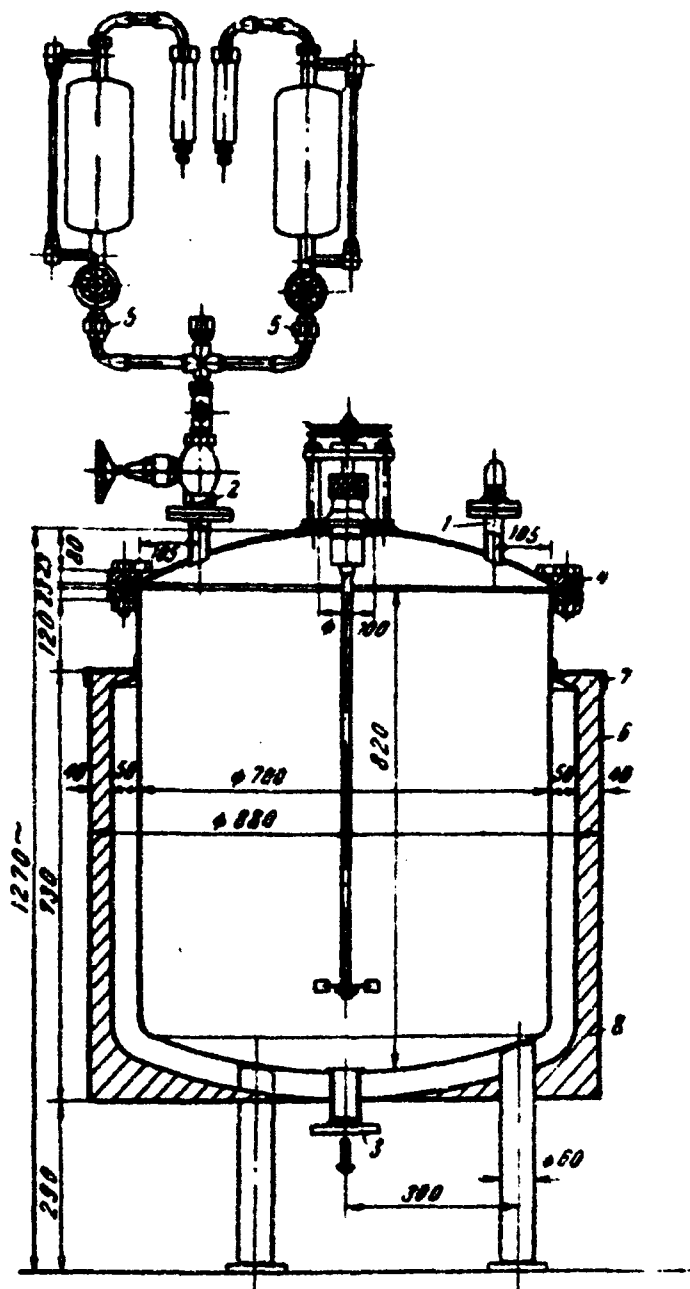


Figure 4. Cross-section of 300-liter cultivator with stationary receptacles for supplements.  
 1 - Air inflow; 2 - valves for introduction of supplements;  
 3 - drain pipe; 4 - brackets for fixing lid to body; 5 -  
 socket joints; 6 - outer jacket; 7 - jacket cover; 8 -  
 glass wool for heat insulation

of the medium blades, called cutters, are placed in the apparatus, and they form a vortex and promote better turbulence of the liquid.

However, some authors (46) consider that more advantageous conditions for the aeration of growing cultures are obtained by the formation of a whirlpool or eddy (vortex) with the rotary motion of the stirrer in the apparatus without the blades (vortex system). The authors believe that with this the air is dispersed in very tiny bubbles at the top of the whirlpool. At the same time the vortex system is more economical in the sense of energy consumption since here, in order to support the movement of the liquid, there is needed only energy to maintain the whirlpool against the static condition of the surface of the liquid. No less important an advantage of the vortex system, in Chain's opinion, is the almost complete absence of foam formation even when there is a large quantity of the substances which produce this.

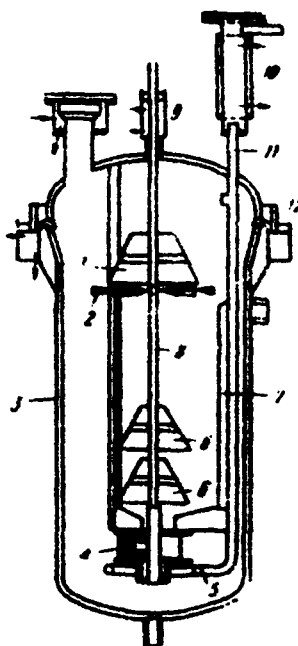


Figure 5. Details of interior fixtures of laboratory type of cultivator.

1 - Cone of foam extinguisher; 2 - plate of foam extinguisher; 3 - body of cultivator; 4 turbine of agitator; 5 - blade openings; 6 - agitator cone; 7 - blade; 8 - agitator shaft; 9 - gasket; 10 - filter for incoming air; 11 - tube leading air into cultivator; autoclave lock

For a long time the development of antibiotic production was delayed by the problem of sterilization of huge volumes of air which was bubbled through the nutrient medium at high velocity under pressure (4).

This problem has now been successfully resolved. The air, which goes into the cultivator, is released from the microorganisms by passing through special filters containing glass cotton and activated charcoal (3). In addition, large-sized porcelain filters with large pores of  $1.5 \mu$  are used (40). The air is conveyed onto the filter by compressors under pressure. Even in the compression process, as a result of strong thermal action on the air, the bacterial contamination in it is impressively reduced (4); thus, the air passes from the compressor onto the filter considerably purified. The filters are periodically sterilized with steam.

Air sterilized in such a manner is bubbled through the fermentor and is discharged through the drain pipe. In work with pathogenic microorganisms the exhausted air is passed through heat where it is decontaminated before it is discharged into the atmosphere (60).

The rest of the fixings of the cultivator consist of appliances which record the temperature, pH of the medium, turbidity, amount of bubbled air, level of medium in the apparatus, and level of foam formation.

Modern fermentors are equipped with devices which not only assist in recording but also in automatically regulating the basic criteria of the technological process and this facilitates stability in cultivation conditions and, consequently, a uniform yield of microbial material.

Mechanical foam extinguishers are used to prevent excessive formation, or substances are added which check it. In the opinion of investigators, the mechanical method is the more desirable.

Of makes which have been exhibited the foam extinguisher described by Humfeld is effective (61). It appears as a disc with blades arranged radially and a cone built into the shaft of the mixer. With excessive formation the foam rises to the level of the extinguisher, is caught by the blades and thrown in the direction of the walls along which it again trickles into the liquid. The foam, which rises to the level of the blades, is forced to the bottom of the rotating cone. This foam extinguisher, according to the data of Heden and associates (47), is effective even with the excessive foaming which occurs in penicillin cultures (Figure 5).

In antibiotic production substances are often added which prevent foam (castor oil, sunflower oil, soybean oil).

lard, and other products). Oily substances, which are added to the apparatus, form a film on the surface of the nutrient medium which also prevents foam formation. Devices have been developed which allow automatic addition to the cultivator of the mentioned substances when there is extreme foam formation. Similar devices have been described in the work of Echevarria (62). The essence of the device consists of the fact that the foam brings the electrodes in the electrical circuit together, as a result of which the device goes into action, providing the foam extinguisher in a dispersing state to the cultivator.

As it has already been mentioned, to reduce possible contamination in the cultivator screw connections, washers, etc are used only in extreme cases, and welded connections are used instead. However, the principal sources of contamination, as Foster has shown (4) are the various subcompartments, gaskets, and recording devices which are difficult to completely sterilize by steam. This pertains first of all to the most susceptible connection - the gasket of the agitator.

The manufacture of gasket packings, which are impervious to bacteria, for the rotating shaft is one of the most difficult problems to solve.

Of the existing makes the best is thought to be the gasket with the steam shut-off and graphite-rubbed surface (47).

Because of the double steam-proof surfaces between the graphite and rustproof steel and the possibility of keeping optimum pressure in the trap of the gasket during the entire cultivation period, the probability of contamination of the contents of the fermentor and the spread of bacteria into the neighboring area is practically eliminated.

The most dependable from the point of view of airtightness is considered to be the cultivator constructed with enclosed electric motors, which allows a system to be formed that is completely isolated from the external environment. Not long ago the English Minister of Supply told of such a type of patented apparatus for the study of microbiological processes (63).

In this apparatus the rotor of the electric motor is located on the shaft of the agitator and is covered by a cylinder of rustproof steel. The winding of the stator is put on the outside of the cylinder. Aeration is accomplished also along a closed system by means of the continuous circulation of gas through the medium. Such an apparatus completely eliminates contact with the outside atmosphere and, consequently, also removes the danger of

contamination from without or the escape of microbial material into the outer atmosphere.

For greater assurance from possible contamination of the cultivator air conditioning, which is free from bacterial contamination, is recommended for buildings of industrial enterprises dealing with microbiological problems (4). This is achieved by making the structures airtight and outfitting them with filter-ventilating systems. In doing this, according to Grenfell, Legge, and White (64), special care must be given to the room where the initial sowing of the original culture is prepared. The "seeding" room, like the viral laboratory, must be equipped with compartments for the work. The air, which goes into the room, is preliminarily purified in the filter-ventilating installations, and in the room itself it is disinfected with the help of ultraviolet lamps.

The technology of submerged cultivation in an apparatus is composed of several stages (3, 40, 46).

The first stage includes the preparation of the seeded material by cultivating the original culture on plates or in bottles. Simultaneously the small (seed) fermentor is prepared: sterilization of the nutrient medium and the apparatus itself.

The second stage provides for the cultivation of the seeded portion of the organism in the small fermentor. From the small seed fermentor the culture, when it reaches the logarithmic phase of growth, is transferred along the pipe-line into the intermediate cultivator or directly into the main recovery cultivator, where further cultivation takes place but now in rather larger volumes, and this is the third and last stage of cultivation (Figure 6).



Figure 6. View of cultivation department of industrial microbiological research.

The most important factor, which provides rapid and maximal growth, is the amount of culture seeded in the apparatus or the magnitude of the seeded dose.

In accordance with the data of Jacobs, Wright, and Hildebrandt (3), the seeded dose of the culture usually consists of 5 - 10% the volume of the cultivator. Using these figures, the necessary number of fermentors can be calculated and also the likely number of stages in the technological process in the cultivation department of industrial concerns.

An approximate scheme of biomycin production is illustrated in Figure 7.

The duration of the cultivation cycle is dependent on the properties of the microorganisms and the cultivation conditions (nutrient medium, aeration, etc).

At the end of the cultivation the finished product is poured into containers and proceeds along its way, and the apparatus is sterilized and filled with medium; after the seed culture is placed in it a new cultivation cycle begins.



Fig. 7, KEY:

- 1) Nutrient medium
- 2) foam extinguisher
- 3) glucose
- 4) peptone
- 5) meat extract
- 6) tank of nutrient medium
- 7) tank of foam extinguisher
- 8) sterilizer for foam extinguisher
- 9) steam
- 10) foam extinguisher
- 11) seeding H<sub>2</sub>O
- 12) air
- 13) steam H<sub>2</sub>O
- 14) fermentor
- 15) culture outlet
- 16) air
- 17) steam or H<sub>2</sub>O
- 18) H<sub>2</sub>O outlet
- 19) air or CO<sub>2</sub>
- 20) steam or H<sub>2</sub>O
- 21) fermentor
- 22) H<sub>2</sub>O culture outlet
- 23) tank for intermediate product
- 24) steam or H<sub>2</sub>O
- 25) fermentor
- 26) air
- 27) filter
- 28) compressor
- 29) cooler
- 30) filter
- 31) sterilized air

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## METHODS OF CONTINUOUS CULTIVATION OF MICROORGANISMS

In the opinion of the foreign specialists (59, 60), cyclic cultivation of organisms under industrial conditions has a number of disadvantages. This primarily is connected with the limited obtainment by the growing culture of nutrient substances, since the nutrient medium in the cultivator is not renewed during the entire cultivation cycle. Metabolic products gradually accumulate in the medium, and they inhibit growth and development of the culture (51). Cyclic cultivation, in addition, encumbers the activities of the entire business, since some delays in production may be created as a result of "intermittent" returns in production.

Since the 20's of the present century individual authors have tried to solve the question about replacing static methods of cultivation with dynamic ones in order to maximally exploit the ability of microorganisms for multiplication. In recent years this idea has taken roots. Monod (49), Novick and Szilard (65), Maxon (66), and other authors have worked out theoretical bases for the continuous cultivation of microorganisms and have constructed a number of units for this purpose.

The substance of the continuous cultivation method is that fresh nutrient medium is constantly being added to the growing culture, and a definite amount of the culture is continually being removed from the apparatus. This makes cultivation possible without subculturing for a long time. In the continuous cultivation process a dynamic equilibrium between the amount of inflowing medium and the amount of cultivated culture can be established by the degree of inflow of a certain amount of nutrient medium into the fermentor. This equilibrium is maintained at a constant level as a result of the addition of fresh medium to the fermentor and the removal of an equal volume of the developed culture.

By providing the necessary rate of inflow of nutrient substances it is possible to keep the multiplication of organisms at a constant level - for example, in the logarithmic phase of growth - and by such a means to continuously obtain the most useful growing "young" culture. The most important theoretical basis to prove the possibility of continuous cultivation was established by Monod (49). He showed that the dynamic equilibrium, which is established between the inflow of fresh medium and the multiplication of organisms, has a stability and is automatically renewed if for any reason there occurs a temporary var-

lation in the established rate of inflow. Therefore, Monod has called such an equilibrium a stable state of the culture.

Later on these concepts were developed in the works of Herbert, Elsworth, Telling (60), and others. The authors demonstrated that a stable state of the developing microbial culture was possible within a broad range of the inflow rate. The producing capacity of the fermentor, which would be equal to the bacterial concentration at the flow rate, rose with an accelerated rate of flow; however, this increase was not limitless. With the attainment of a critical flow rate the concentration of organisms in the culture fell sharply. Thus, for a stable state of the culture a certain relationship was necessary between the growth rate of the microorganisms and the flow rate, i.e., the inflow of fresh nutrient medium in a known range of deviation to either side. But if the culture was diluted again with incoming medium at a speed exceeding the possible rate of growth of the bacteria, then they were finally "washed out" of the culture tank. A mathematical relationship was established between the basic factors causing the process of continuous cultivation.

Foreign investigators, working in the field of continuous cultivation of microorganisms, have reported a number of advantages of this method over the cyclic, particularly the possibility of accurately regulating the cultivation conditions. As Maxon has shown, it is possible to obtain large volumes of a uniform culture in the necessary concentration. Growth with continuous cultivation has a greater homogeneity than with the cyclic.

By culturing with the flow method the probability of microbial mutation is reduced to a minimum, since cultivation takes place in a constantly renewed medium and with the regular removal of the growing culture. Therefore, even if a mutant does appear, most of the changed cells will be removed before they can multiply (60). However, this position has been disputed by some authors (65, 69, 70, etc).

A second advantage of the continuous method consists of the fact that such a process is somewhat easier to make automatic, which is even facilitated by the ability of the culture for self-regulation (71). Finally, the yield of the continuous method of cultivation is incomparably higher than that of the cyclic. Thus, for example, Barnes and Dewey (51), cultivating a virulent strain of Flexner dysentery by the continuous method, recovered in 2 1/2 - 3 hours a concentration surpassing by 8 - 10 times that of an identical culture grown under the usual conditions in

18 - 24 hours. However, such an unbelievable increase in the yield in a short period of cultivation leaves one uncertain as to the validity of these data.

Continuous cultivation is time-saving due to the elimination of nonproductive operations which are so numerous in the cyclic method (sterilization of the apparatus, pouring of the medium, refrigeration, etc). Therefore, continuous cultivation is now being extensively used in the fermenting industries. It is especially widely used in the yeast industry (72).

In Rosebury's book, "Peace or Pestilence" (2), he mentions units of continuous cultivation developed at Camp Detrick in which it was possible to produce about a pint (0.568 liter) of brucella culture every eight hours.

As negative aspects of continuous cultivation of microorganisms, some authors have pointed out the instability of the cultivated strain with prolonged cultivation (51) and also the danger of contamination. Maxon (66) maintains that if with the cyclic method contamination results in a loss, let us assume, of one batch (fermentor), then in continuous flow the losses may be considerably greater and may even lead to a suspension of the entire operation. However, it will not be superfluous to mention that a unit of the industrial type of Herbert, Elsworth, and Telling' (60) worked for four months continually without contamination.

Let us dwell briefly on the basic elements of construction of appliances and units for continuous cultivation of microorganisms (according to the data of the foreign literature).

Felton and Dougherty (73) in 1924 studied the influence of growth rate on virulence with the cultivation of a pneumococcus strain in a semi-continuous unit. The unit consisted of a tank for the nutrient medium, a holding tank for the culture, and a tank for the final product. The nutrient medium flowed into the holding tank by drops under the control of an electromagnetic relay. When the nutrient medium with the growing culture reached a certain level in the tank, it flowed into a tank for the finished product.

This elementary unit was improved later on, but the principle on which it was based has remained the same. The improvement resulted in better aeration of the culture and a revision of the device which regulated the cultivation process.

In units proposed by Novick and Szilard (65), Rotman (74), Kubitschek (75), Fox and Szilard (76), and others, aeration was accomplished by bubbling air, which was

supplied from underneath, through the culture tube. In the unit of Barnes and Dewey (51), along with the supply of air to the culture flasks they were constantly shaken to provide better aeration. In the apparatus of Monod (49) aeration was accomplished by rotating the culture container. With this type of aeration foam formation was excluded. As an improvement in the unit Monod exhibited the apparatus of the Swedish investigators Heden, Holm, and Malmgren (77), which provided the recovery of large volumes of microbial material with sterile conditions of the cultivation process. A general disadvantage of all these units, as the authors themselves have admitted, was the difficulty in regulating the cultivation process, since the growth rate here was determined only by the speed of the media supply without a clear accounting and control of the bacterial concentration in the culture.

In the more modern units of Anderson (78), Northrop (70), and Mitchell and Plummer (79) automatic regulation of the process has been realized. The action of the regulating devices is based on the principle of a photoelectric colorimeter, which constantly maintains the culture in the apparatus in the logarithmic phase of growth. With the achievement of the necessary concentration of organisms a certain amount of nutrient medium is automatically fed into the cultivator, and a corresponding amount of culture is removed. The disbursement of the medium is controlled and regulated by a special auxograph.

Finally, we will briefly describe the unit of Herbert, Elsworth, and Telling (60), who have already had experimental production (Figure 8).

The unit consists of a reactor, two tanks for nutrient media, and a tank for the discharge of the finished microbial culture. The entire unit is made of rustproof steel. The working capacity of the fermentor is 20 liter, and it is of serial construction. Agitation and aeration are accomplished by a plate stirrer with blades, and the aprons and sparger for the air are located directly under the stirrer, i.e., exactly as in Chain's unit (46) and others.

The tanks for the nutrient medium are parallel to one another and are connected, and they are used alternately for feeding the medium into the reactor (CV). The working capacity of each tank is 300 liter. Sterilization of the medium is accomplished in them.

The air entering into the reactor and the exhaust air are sterilized by heat. The inflow rate of the medium and the rate of the air current is regulated by rotameters and controlled by special stopcocks. The culture enters

the measuring tanks  $M_1$  and  $M_2$ , which are parallel to one another and connected; a second check of the flow rate is made in them. From the measuring tanks the culture goes into the main tank H.

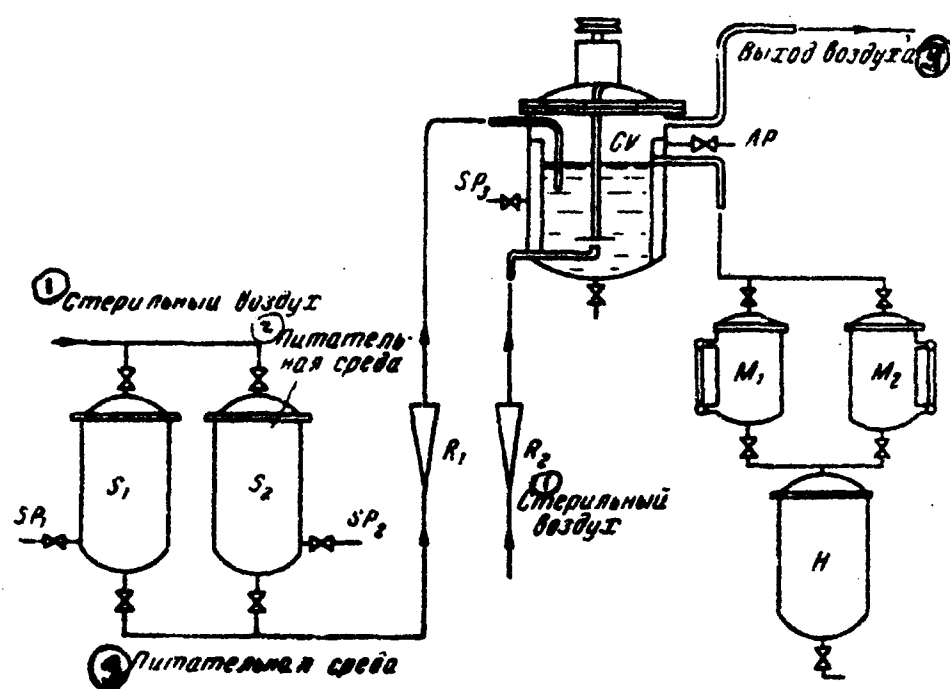


Fig. 8. Scheme for continuous cultivation in unit of Herbert, Elsworth, and Telling.  
1-Sterilized air; 2-Nutrient medium; 3-Air exhaust

The point AP on the diagram signifies the place where the seeded material and the foam extinguisher are introduced; SP<sub>1</sub>, SP<sub>2</sub>, SP<sub>3</sub> - taps for sampling. The temperature in the fermentor is regulated by heated water flowing through the coils of the reactor (not shown on the diagram).

In the fermentation industries it is sometimes desirable to accomplish continuous fermentation with the use of several units joined in a series. In this case part of the culture from the last unit is used as a seeding dose which is fed to the first unit. The use of several units in the diagram is continuous cultivation as Maxon has demonstrated it (66) and permits the producing capacity of the unit to be increased.

\* \* \*

Some aspects in the present section on the mass recovery of microbial material refer to common bacterial forms of microorganisms and fungi. The production of microorganisms and products of microbial fermentation in recent years has noticeably expanded in many countries primarily due to an increase in the manufacture of antibiotics. Thus, according to the data of Perlman and Tempel (80), in 1952 the productive output of businesses of the USA, who were marketing chloromycetin, terramycin, and aureomycin, was estimated to be 24 tons.

Recently reports have appeared in the foreign literature about the possible cultivation of viruses and rickettsiae by the submerged method in an apparatus. A liquid nutrient medium consisting of tissue cultures is used for this. The investigations of McLimans, Davis, Glover, and Rake (81) have shown that in the cultivation of cells of mammals the cells easily proliferate in five liter volumes of serous nutrient medium in standard fermentors with the agitation of the cultures. Analogous data on the cultivation of mouse fibroblasts were obtained by Shannon Danes (82) in a specially constructed apparatus with a capacity of 250 ml. Very recently Ziegler, Davis, Thomas, and McLimans (83) reported on the successful cultivation of cells of mammals in 20-liter steel fermentors.

The authors have shown the potentiality of submerged cultivation methods for the receipt of high concentrations of virus material for vaccine production. Thus the principle of submerged cultivation can also be applied to the cultivation of viruses and rickettsiae in mass quantities.

## PROBLEM OF DESSICATION OF MICROORGANISMS

Analyzing the principles of selection of potential biological agents of warfare, Rosebury gave special attention to preserving the activity of organisms which can be used as a bacteriological weapon. It is not accidental that most of the pathogenic microorganisms, as it is known, belong to the category of nonresistant biological agents. It is especially difficult to preserve microorganisms for a long time outside the body, i.e., under inadequate conditions which differ sharply from natural existence.

Organisms are especially sensitive to maintainance in liquid media. This is due to the influence of electrolytes on the cells and also to changes in the media as a result of the metabolic process of the organisms.

The most effective method of long-term maintainance of biological preparations, particularly microorganisms, as experience of the last decades has shown, is drying under special conditions, and it is called lyophilized drying.

Like all biological agents, microorganisms are extremely sensitive to drying, i.e., the loss of intracellular moisture which makes up more than 90% of the contents of any cell. Especially sensitive to drying are the vegetative forms of organisms; the more resistant are some rickettsiae, viruses, and spore-forming microorganisms. Under the influence of sunlight many microorganisms, suspended in the air, are killed in a few minutes. The rapid death of the organisms is caused, on the one hand, by the bacteriacidal influence of ultraviolet radiation on the cells and, on the other hand, by the intensive evaporation of cellular moisture due to the thermal effect of irradiation.

A decrease in the moisture content of plasma cells causes an increase in the concentration of salts, and this favors the formation of conglomerates of the protein molecules. According to the extent of the rise in the salt concentration the protein molecules are subjected to irreversible aggregation (denaturing) (84). This is why the attempts at simple drying of organisms and other protein substrates, which many authors have undertaken, have terminated in failure as a rule. These attempts result in microbial cultures being dried in a dessicator over chemical absorbers ( $P_2O_5$ , KOH) or in drying cabinets with heat. Favorable results are obtained in this way only with the drying of spore forms.

At the beginning of the 20<sup>th</sup> century Shakell (1909)

submitted the principle of a new method for the drying of biological preparations - drying from the frozen state.

At first these experiments did not attract the attention of investigators, and only later, especially during the First World War, when blood sera and plasma for transfusions could be prepared and stored for a long time by the method of Shakell, Flosdorf, and Mudd (85), did this method receive general recognition and become quickly developed.

At present the method of Shakell is broadly used in many branches of industry (pharmaceutics, medicine, antibiotics, fermentation, food, etc) for the conservation of various protein substrates. In the literature it is often called the method of lyophilized drying. The basic principle of lyophilized drying is the preliminary freezing of the material to be dried. The drying process includes the removal of moisture from the frozen material by the immediate conversion of ice into vapor, omitting the liquid phase. Therefore, the protein substrate or solution can be dried without aggregation of molecules and the action on them of high concentrations of salt (84), and this protects the protein from coagulating and denaturing. The drying substrate is a spongy mass, the weight of which approximately equals that of the volume of original frozen substance. Thus, lyophilization is nothing but the process of sublimation (85a). Therefore, it is now customary to refer to the method of drying from the frozen state as sublimation drying. But the term "lyophilization" characterizes only the preservation of the original property of the dried material to be dissolved, which points to the undisturbed colloid-chemical structure.

An important condition of lyophilized drying is the need for a vacuum. With the drying of the frozen material under conditions of normal atmospheric pressure the volatilization process can be prolonged indefinitely. But if the frozen material is dried with reduced pressure, the rate of drying rises sharply because with reduced pressure the intensity of water evaporation is increased. With a lowering of the pressure to 4 mm of the mercury column or lower the frozen material can even dry if it is heated a little in order to expedite still more the drying process.

Additional heating of the frozen material in a vacuum does not induce its melting, since this pressure is below the vapor tension of ice at 0 degrees.

Thus, the use of a vacuum with the frozen material allows the drying process to be sharply speeded up.

As it has already been shown, microorganisms bear low temperatures rather well. However, a sudden and sharp



cooling is not a completely harmless act for them. Some bacteria are highly sensitive to sharp fluctuations in temperature. Thus, for example, in the experiments of Hegarty and Weeks (86) a sudden chilling of the growing culture of intestinal bacilli from 37 to 0 degrees led to the death of the cells, and the number of dead cells in the culture was greater than the number of developing ones. Nai, Khayasi, Sato, Okhara, and Nakagawa (87) consider that rapid cooling usually causes marked damage to the cells. This phenomenon, referred to as temperature shock, is observed in most cells of vegetative and animal origin (88). Temperature shock is also observed with the chilling to low temperatures of 0 to -70 degrees (89). The reason for its occurrence has not been precisely established. Smith and Krueger (90) associate the appearance of temperature shock with changes in osmotic pressure in the cells as a result of freezing. The most widespread theory explaining the mechanism of the emergence of temperature shock is that damage to the cells results from the formation of ice crystals with freezing (91, 92, 93, 94, 95, etc). However, this hypothesis does not explain the mechanism of temperature shock with the rapid chilling of cells to 0 degrees without freezing.

The intracellular formation of ice crystals occurs differently. It is usually considered that slow freezing is accompanied by the formation of large crystals which cause a mechanical injury to the cells. With rapid freezing tiny ice crystals form, and trauma to the cells is somewhat less with this. Finally, crystallization can be reduced with extra fast chilling and freezing.

Luyet (91) introduced a hypothesis by which trauma to the cells can be avoided with their freezing. The hypothesis is founded on the works of Tamman and can be generally outlined in the following manner. With the usual freezing water goes from a liquid state into a solid crystalline one, which is the cause for the mechanical damage to the cellular structure of organisms or viruses. However, under certain definite conditions water or liquid can freeze in the form of an amorphous, glassy mass without the formation of crystals. This is the so-called vitrefaction phase.

The crystallization process, according to Tamman, occurs only with a freezing temperature or close to it. With temperatures ranging below this, in a relatively narrow zone, crystallization is impossible. This appears a decisive factor in the ultimate glassiness of the liquid. Therefore, Luyet considers that the death of the cells does not occur with low temperatures if the protoplasm of the

cells is successfully converted into a glassy state and then again into liquid without the formation of crystals within the cells. To achieve this Luyet recommends that the bioagent, which is to be dried, be cooled with maximal speed. By such a means it is possible to "slip by" the critical temperature zone in which the crystallization rate is maximal.

At present most investigators adhere to the point of view of Luyet.

However, as Fry and Greaves properly point out, it is not always possible to freeze material rapidly, especially in a large quantity. To do this it is necessary for the mass of frozen substrate to have excellent contact with the freezing agent, and the temperature in the chamber must reach a very low figure. An excellent drying quality is achieved if the material is frozen at a temperature below the eutectic (94).

Preservation of the organisms during the drying process and after it primarily depends on the nature of the microorganism. Thus, for example, the poliomyelitis virus according to the data of Pollard (96), is extremely sensitive to sublimation drying and with this treatment loses both pathogenic as well as antigenic properties. On the other hand, Elser, Thomas, and Steffen (97) have preserved tetanus toxin dried by this method for seven years, and meningococci and gonococci for 18 years.

In the opinion of a number of investigators, apart from individual properties and peculiarities of the microorganisms, great importance is given in drying to external factors, particularly the influence of the drying medium, age of the culture, concentration of the microbial suspension to be dried, drying regime, and residual moisture (94). A satisfactory adoption of these factors can insure excellent survival during and after dessication of even the most fastidious and sensitive microorganisms.

In most works published in the last 40 years special reference has been made to protective colloids as a suspension medium for drying. Bouillon, serum, blood, or milk are the agents most used (98). Naylor and Smith (99) noted the protective role of gelatin and thiourea, and Fry and Greaves successfully used glucose and lactose as a suspension medium for drying.

Annear (100, 101) reported on favorable results with his dessication of bacteria in a peptone medium and also in cellulose and sodium alginate. Harris confirmed that a medium rich in protein is necessary for the effective drying of viruses. However, the mechanism of action of the protective colloids has not yet been established (89).

For better preservation some authors recommend that filtrates of old microbial cultures be added to the cultures to be dried (before drying). Bergmann, Halleck, and Mechalias verify the data of Hatton and Tierney, Reccord and Taylor about the presence of old incubated cultures in the filtrates as a factor which aids in protecting the lyophilized cells.

Survival of the organisms in the drying process depends also on the concentration of the bacterial suspension. Stamp (103) observed that with a lowered concentration of cells in the suspension the survival rate rose.

Cultures of microorganisms in the logarithmic phase of growth give the best results with dessication, but they should not be too young (99, 94, 100). Finally, the drying routine and the conditions of subsequent storage influence the survival of microorganisms. The available data confirm the unfavorable action of too rapid drying. The maximal death of the organisms is encountered during the first few hours of drying when the most rapid removal of water takes place (Fry and Greaves).

It is recommended that the dessicated cultures be stored without access to air. The optimal condition for this is storage in a vacuum, as experience in vaccine production confirms.

According to the data existing in the literature (89) oxygen shows an unfavorable action on stored cultures. Moisture in the air also acts negatively on dessicated microbial cultures, causing their "humidification" and death of the cells (98).

The influence of residual moisture on the survival of dried bacteria and viruses has been insufficiently studied. Beckett (104) believes that for the successful preservation of viruses there should be less residual moisture than for bacteria. It must be stressed that for the optimal preservation of dried biological material residual moisture must be kept at a minimum, but the cultures should not dry out completely because organisms, in Greave's opinion (84), will survive best if they are not overdried.

## APPARATUS FOR DESSICATION

Many methods and devices exist for the drying of microorganisms from the frozen state. Laboratory apparatus have been described in a number of review articles and monographs (105, 106, 89, 85, etc), so we will not dwell on their features.

Modern apparatus for lyophilized drying from the point of view of construction are divided into two classes: collector and chamber. The first are used in vaccine production, the second in antibiotic manufacture.

The principle of action of the collector dryer consists of the following: the material to be subjected to drying is packed in sterile ampules and undergoes preliminary freezing with the use of different kinds of chilling mixtures. The ampules with the frozen material are attached to the collector apparatus, which is a pipe-line with branch pipes for contact with the ampules. Drying is achieved under high vacuum (50 - 100  $\mu$ ) which is reached by evacuation of the air from the apparatus with a vacuum pump. . Water vapor from the material to be dried is absorbed by chemical absorbers (phosphorus pentoxide, gypsum), or precipitates on condensers chilled by various refrigerants (Freon, ammonia, solid carbon dioxide), or, lastly, it is removed in the atmosphere.

The advantage of the collector apparatus is dependability in the sense of maintaining sterility in the work. However, their productivity is very limited, and it is extremely difficult to dry large amounts of biological material on them. Therefore, chamber drying units are more often used for industrial purposes. The modern industrial vacuum-dryer (39) is a hermetically sealed chamber of a cylindrical or rectangular shape (cabinet). The inside area of the cabinet is completely filled with shelves. Over them are installed coils along which the refrigerant circulates. Receptacles with the material to be dried are placed on the shelves. The chamber is closed, and the air is evacuated with a vacuum pump to the necessary point. The material is frozen at a low temperature, and sublimation is begun with the gradual heating up of the chamber by turning on the steam under the jacket or by especially built heaters (Figure 9).



Figure 9. Type of chamber sublimation dryer.

The control for the temperature in the chamber and in the material to be dried is taken care of by thermometers and thermoelements, and the residual pressure is regulated by vacuum gages. The moisture vapor drawn off by the vacuum pump is collected by condensers of various types and is periodically removed.

The chamber dryers have great productive capacity, but their essential defect is the periodic action and incomplete automatic operation in the drying process. The loading and unloading of the drying material is done manually through the open door of the chamber. With this the vacuum is naturally broken, and the hygroscopic nature of the dried material is sharply reduced. The wetting of the dried biological material markedly cuts the length of storage and causes intensive decay of the microorganisms. In addition, this manipulation of the dried microbial material, as it has repeatedly been observed in the foreign literature, often contributes to the formation in the room of a bacterial aerosol of such sizable concentrations as to represent a great danger to the personnel.

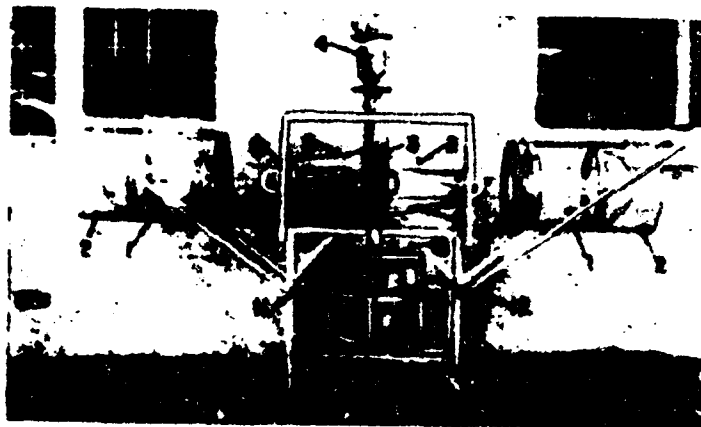


Figure 10. Type of laboratory sublimation dryer with continuous action.

1 - Dome-shaped vessel of organic glass; 2 - plastic framework; 3 - central ring support; 4 - container with frozen material; 5 - nutrient conduit; 6 funnel for nutrient; 7 - mesh band (16 mesh); 8 - heater; 9 - unloading conduit of prepared material; 10 - pulley for convey belt; 11 - condenser; 12 - frame; 13 - connecting pipe for vacuum system.

The cited disadvantages have caused investigators to work toward the development of sublimation drying units of continuous action in which the operation of loading and unloading the product occurs under hermetic conditions. In addition, in the continuous method of dessication, as Fixari, Conley, and Violl (107) have indicated, the operating expenses are impressively reduced.

The standard unit for the continuous drying of

microorganisms is the vacuum dryer constructed in the laboratories at Camp Detrick in 1952 (108) Figure 10).

Investigations on the drying of the microorganism, *Serratia marcescens*, which was performed in this dryer, gave satisfactory results (109).

The unit is constructed in this way so that the loading into the drying chamber of the material, frozen in the form of granules, and the unloading of the dried material from it occurs without interruption of the drying process.

The essential parts of the dryer are: a horizontal vacuum-drying chamber in which two rotating cylinders support a conveyor belt, heater, device for supply and removal of dried material, refrigerating system, vacuum pumps and control gages which are built in a special panel.

The microbial material in the form of a suspension is fed by drops through a square-shaped needle into the bottom of a Freon bath where it is frozen in the form of granules. The size of the granules depends on the size of the drop, which is regulated by the rate the material is fed in.

The frozen granules are delivered through a special loading unit into the chamber on a moving metal belt. Moving on the belt, as on a conveyor, from the first to the second cylinder, the microbial material is dried and then with the help of the conveyor device it is collected in the receptacle for the finished product (Figure 11).

The attempts of some foreign investigators to use the method of heat drying for the dessication of microorganisms have attracted attention. Mazur and Weston (110) have dried spores of the fungi *Aspergillus* and *Pestalotia* with the help of a dispersion dryer. In these dryers the dessication was accomplished in a current of warm air. The biological material was supplied through a sprayer into the chamber and fell in the current of hot air. The sprayer pulverized the material into tiny droplets, which sharply increased the total surface of the drying material and evaporation took place from the surfaces. Due to this, dessication was accomplished almost instantly, and the temperature of the material did not have time to rise to a higher level.

In the experiments of the cited investigators it was established that heat drying with spraying significantly surpassed the sublimation method in effectiveness, since in the first case 98% of viable cells were preserved, and in the second case only 10%. The authors recommend that the method of spray drying be used for the dessication of resistant forms of microorganisms, since, apart from ef-

fectiveness, the given method is rather simpler than the sublimation and is more advantageous economically.

\* \* \*

Thus, modern microbiological science and practice offer enormous potentials for the mass production of micro-organisms and



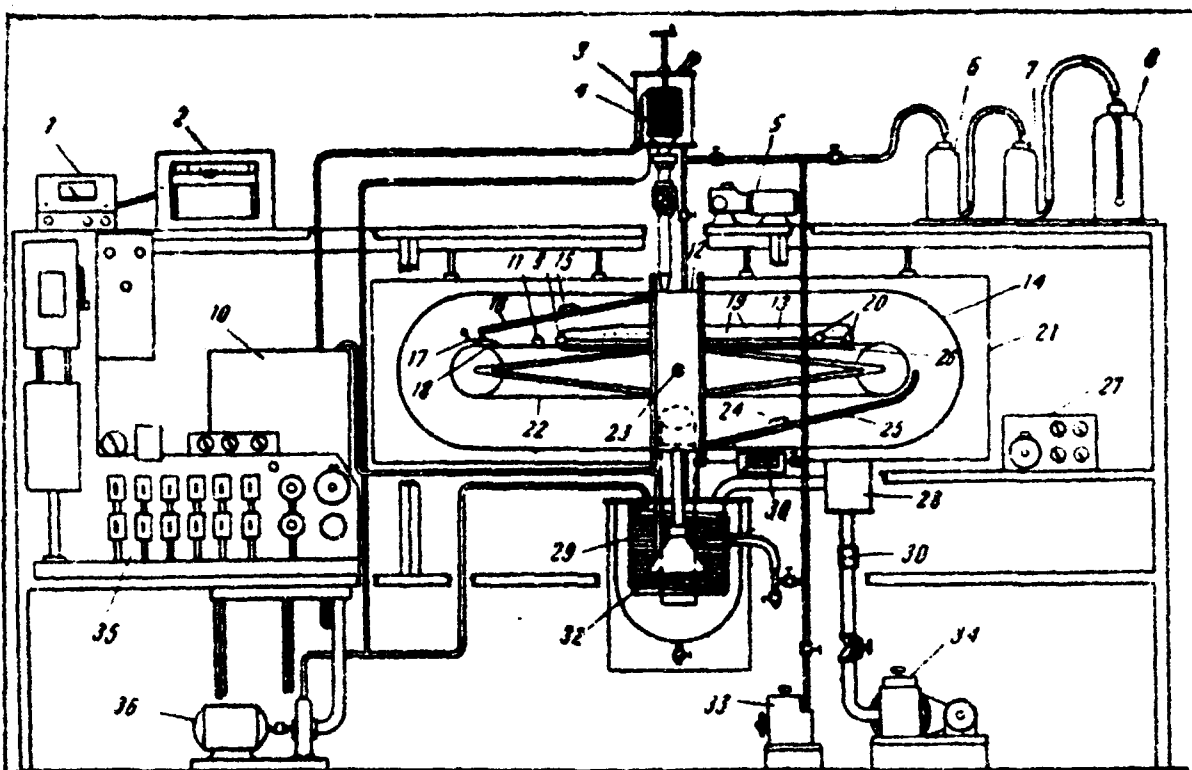


Figure 11. Scheme of construction of dryer of continuous action and accessory parts.

1 - Alphanatron vacuum meter; 2 - recorder for registering vacuum; 3 - feeder; 4 - container with frozen material; 5 - speed control; 6 - filter-dryer; 7 - filter with fiber glass; 8 - filter with sulfuric acid; 9 - thermometer; 10 - refrigeration receptable; 11 - regulator; 12 - coil; 13 - heater and heat reflector; 14 - container of organic glass; 15 - vibrator; 16 - nutrient trough; 17 - loading device; 18 - regulating plate; 19 - heater; 20 - thermometer; 21 - plastic framework; 22 - mesh belt; 23 - pulley for conducting belt; 24 - vibrator; 25 - unloading trough of finished material; 26 - heat reflector; 27 - panel board for heater; 28 - filter; 29 - collector; 30 vacuum gauge of drying chamber; 31 - connecting piece; 32 - condenser; 33 - auxiliary vacuum pump; 34 - main vacuum pump; 35 - panel board; 36 - Brown circulation pump.

products of microbial fermentation. In recent years there has been extensive development in the production of antibiotics, vitamins, yeasts, and some microbial enzymes.

Thus, for example, only in the United States of America, according to a report of the Commerce Department of the USA (111), did the production of some products of microbial fermentations in 1954 - 1955 command the following figures (Table 4).

Table 4

① Продукт ферментации	② Год	③ Производство в фунтах	④ Стоимость в долларах
⑤ Пенициллин и его соли	1954	631 000	53 630 000
	1955	455 000	43 980 000
⑥ Стрептомицин	1954	141 000	5 497 000
	1955	154 000	5 127 000
⑦ Дигидрострептомицин	1954	446 000	19 476 000
	1955	369 000	16 318 000
⑧ Неомиксин и его соли	1954	15 000	4 563 000
	1955	15 000	5 222 000
⑨ Остальные антибиотики	1954	597 000	157 278 000
	1955	579 000	171 745 000
⑩ Антибиотики для подкормки животных	1954	479 000	25 871 000
	1955	520 000	26 105 000
⑪ Общее количество для применения в медицине и ветеринарии	1954	1 837 000	240 128 000
	1955	1 572 000	242 372 000
⑫ Рибофлавин для применения в медицине и ветеринарии	1954	278 000	6 100 000
	1955	136 000	3 349 000
⑬ Витамин B <sub>12</sub>	1954	422	18 894 000
	1955	488	20 614 000

① Fermentation product    ② Year    ③ Production in pounds    ④ Cost in dollars

Penicillin and its salts ⑤  
Streptomycin ⑥  
Dihydrostreptomycin ⑦  
Neomycin and its salts ⑧  
Other antibiotics ⑨  
Antibiotics for animal  
fodder ⑩  
Total amount for medical ⑪  
and veterinary application  
Riboflavin for medical and  
veterinary application ⑫  
Vitamin B<sub>12</sub> ⑬

<sup>1</sup>One American pound is equal to 0.453 kg (A.F.)

Even in 1953 there was an overproduction of penicillin and streptomycin in the USA. Therefore, a significant part of antibiotic production was switched over to a supply of antibiotics for the use of animal fodder (80).

The presented data illustrate to some degree the possibility of modern microbiological production. Taking into consideration the viewpoints and statements of foreign investigators, it can be assumed that the principles on which this kind of production is based may be used by the aggressive military circles of the imperialistic countries to build plants for the manufacture of the bacteriological weapon.

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## CHAPTER IV

### PRINCIPLES OF SELECTION AND POSSIBLE AGENTS OF BACTERIOLOGICAL WARFARE

#### REQUIREMENTS OF AGENTS OF INFECTIOUS DISEASES OF MAN AND ANIMALS WHICH CAN BE USED AS A BACTERIOLOGICAL WEAPON

In the 1930's in the foreign literature, devoted to the problem of bacteriological warfare, isolated statements began to appear concerning the requirements made of its possible agents.

It was very evident that among the colossal number of microorganisms found in nature the attention of specialists turned only to those which had pathogenic properties for man, animals, and plants.

In the opinion of all foreign authors who have discussed this question, the basic requirement made of possible agents of bacteriological warfare is the ability to cause infectious disease, i.e., pathogenicity.

If the concept of pathogenicity is to a certain extent sufficient for the demonstration of disease-producing organisms, then the presence of pathogenicity does not satisfy the basic requirement in the determination of possible



agents of the bacteriological weapon.

For potential agents of bacteriological warfare, in the opinion of foreign authors, the salient criterion is the degree of pathogenicity of microorganisms, i.e., virulence. Therefore, the presence in the agent of highly manifested virulence is a necessary requirement made of it as a possible agent of bacteriological warfare. In this respect we have the many observations of foreign experts. Even in the 40's many foreign authors, discussing the list of requirements made of possible agents of bacteriological warfare, wrote of the necessity of using strains of microorganisms with high virulence (1, 2, 3, etc). In recent years this opinion has been more and more accepted, and an account of the requirements of agents of bacteriological warfare usually begins abroad with a consideration of questions of virulence of the agent (3, 4, 6).

Worthy of attention is progress abroad in the field of bacterial genetics, directed at the artificial change in virulence and in other properties of microorganisms.

Data of recent years in the field of selection and the biochemistry of microorganisms confirm that, in addition to avirulent, vaccine strains of pathogenic microorganisms, strains can be obtained which have increased virulence. Thus, Zelle, Lincoln, and Young (7) reported in 1946 on obtaining a method of selection of a highly pathogenic strain of the anthrax agent.

Knight (8), studying cultures of the tobacco mosaic virus, established that these strains differed by their varying content of 16 out of 19 amino acids present. The 14DI mutant, obtained by Knight from a strain of the tobacco mosaic virus, was distinguished from the original strain by other constituents of valine, lysine, and glutamic acid, and at the same time it also differed sharply in virulence. In contrast to the original virus, strain 14DI killed young plants of Turkish tobacco and tomatoes.

Using the principle of transformation, Griffith (9) converted the noncapsulated and avirulent strain of pneumococcus into a virulent, capsulated one.

Investigations on transformation received a scientific foundation with the establishment of Avery, MacLeod, and McCarty (10) of the chemical nature of the transformation source, which proved to be desoxyribonucleic acid (DNA).

In experiments with pneumococci it was established that the frequency of transformation depended not only on the presence and influence of DNA but also on the physiological state of the bacterial cell in relation to the cultivation conditions.

At a later time still another phenomenon, called transduction, was described which verifies the importance of DNA. The essence of the phenomenon is contained in the ability of some phage strains to transmit new properties to bacteria.

Developing the phenomenon of transduction, Freeman and Groman (11) used a phage isolated from a lysogenic culture to convert non-toxic strains of diphtheria bacilli into toxic ones.

At the present time the mechanism of action of DNA on the microbial population has been extensively developed.

Braun and Whallon (12) established that the greatest number of changes in the population arose only if a homogeneous strain were cultivated in a medium containing DNA isolated from a heterogeneous strain.

Schaeffer (13) showed that there was possible transformation between strains of one origin (homotransformation), between different strains of the same species (isotransformation), and between strains of different species (heterotransformation), although the latter is observed very rarely.

Braun, Firshein, and Whallon (15), studying the effect of desoxyribonuclease on bouillon cultures of brucella containing DNA, established that with the addition of an enzyme there occurred a rapid change in the population and a transfer of the M- and R-forms into the virulent S-forms.

Results of the experiment showed that the breakdown products of DNA depressed growth or destroyed the cells, which did not occur in the S-form. A similar selective action was shown by a compound with the chemical structure -- 6-furfurylaminopurine or kinetin -- a division factor of the plant. As for DNA and desoxyribonuclease, then they, as investigations have shown, not only promote the formation of virulent cells in vitro but also show an influence on the virulence of the S-forms of some organisms in vivo.

The American virologist, Fraenkel-Conrat (16), successfully divided the tobacco mosaic virus into its constituent parts -- protein and nucleic acid -- and then again combined them, receiving a substance which had all the properties innate to the given virus.

Although the actual possibility of synthesis of microorganisms is still very remote, nevertheless the artificial synthesis of protein substances may perhaps be used to obtain various components of microbial cells and also new toxic substances unknown at present.

In recent works of Fraenkel-Conrat (16) it is shown that viral fragments also have infectious capabilities.

ties.

In addition, in recent years a number of papers have appeared in the foreign literature on the recovery of microorganisms by mutagenesis which have increased resistance to antibiotics.

Hotchkiss (17) grew out a strain of penicillin-sensitive pneumococcus on extracts containing DNA isolated from a strain of pneumococcus resistant to penicillin. His new pneumococcus variant was resistant to penicillin.

It is characteristic that this conversion was performed in the absence of an antibiotic in the medium, and resistance is a hereditary adjunct.

The presented data show the great possibilities revealed to investigators in the realm of variabilities of microorganisms in connection with the exposure of genetic processes.

On the basis of the stated facts Rosebury's (18) statement becomes more definitive that for purposes of bacteriological warfare microorganisms can be used with artificially changed properties, for example, with increased virulence.

This is accompanied by a number of comments by foreign specialists, which confirm that many microorganisms that are pathogenic for man and animals and have high virulence, all the same do not satisfy the requirements made of them as agents of the bacteriological weapon, because the diseases caused by them are characterized by a comparatively mild course (2, 18). Thus, for example, in the opinion of foreign authors, the agents of food-borne salmonella infection, which does indeed possess high virulence, could scarcely be used as a bacteriological weapon, because the sicknesses caused by them progress rather mildly, are of short duration, very rarely terminate fatally, and do not actuate invalidism. A similar opinion has been expressed as well in respect to the virus of lymphocytic choriomeningitis, Friedlander's bacillus, and some other microorganisms (2).

Consequently, along with virulence, in the opinion of foreign specialists, the factor defining the potential use of microorganisms as a bacteriological weapon is the nature of the disease caused by them or, as Rosebury and Kabat (2) determined, the effectiveness of action of the agent; by this is understood the duration of the incubation period, severity and length of the course of illness and convalescence, and mortality. For very obvious reasons most foreign authors prefer in this respect microorganisms which cause severe diseases with high fatality (4 and others. In addition, some military specialists in the USA do

not reject those agents which cause diseases with negligible fatality but which are characterized by a prolonged course and incapacitation of the patients. In reference to this General Bullene (20) stated the following: "The choice of bacteria which can be used during biological warfare depends on the purpose for which it is used. If the objective is to kill a large number of people, then use the agents of such diseases as plague, typhus, cholera, smallpox, etc. But if the intention is only to make people sick and not to kill them, then the agents of tular-emia and brucellosis are more suitable."...Attacking op- tionally can be aimed at the annihilation of people, but if this provokes disease, it will be quite enough to de- prive people of fighting efficiency and working ability. It will also help to increase the man-hours and to over- flow the hospitals."

In an appraisal of the agents of infectious diseas- es as possible agents of the bacteriological weapon much attention is given in the foreign literature to the com- municability of the illnesses caused by it, i.e., the abil- ity for epidemic spread. As a matter of fact, the view- points are by no means unified. Some foreign authors as- cribe special importance to communicability, seeing in it the basic feature of the bacteriological weapon (19), and others, on the other hand, consider that communicable dis- eases cannot be anticipated in bacteriological warfare since they have a pronounced retroactive effect (?). Thus, the character of the illness caused by the agent or the ef- fectiveness of its action is an extremely important re- quirement made of the possible agents of the bacteriologi- cal weapon.

Another necessary requirement, broadly discussed in foreign literature, is the feasibility of cultivating the agents in large quantities. Let us assume that the micro- organisms have the predisposition to cause severe infec- tious diseases with high mortality, but if methods have not been developed for their mass production, they will not satisfy the requirement made of possible agents of the bac- teriological weapon (4, 6, 18).

This is the ultimate requirement, in the opinion of foreign authors, because agents can be used as a means of bacteriological warfare only if it is possible to procure them in large quantities. The agents, which are difficult to cultivate and which may not be obtained in large amounts, cannot, in their opinions, be potential agents of the bac- teriological weapon.

Along with the enumerated requirements, great im- portance is given to the degree of resistance of the agents to various influences of the external environment, both in

the storage of the prepared material as well as after application. It is well known that many vegetative forms of bacteria have negligible stability and perish very quickly with unfavorable cultivation conditions. In the opinion of Rosebury (2), Wiesmann (19), and other authors a preference will be given to those agents which are able to preserve their viability for a long time. On the one hand, this facilitates the building of reserves and, on the other hand, the locality where these agents are applied will be hazardous for a long time in the sense of infecting man and animals.

Stable forms of organisms are also more resistant to the effect of various disinfecting agents, which (in the opinion of the indicated authors) might greatly hamper the conduction of defense measures.

Therefore, in a consideration of these questions in the foreign literature it is not incidental that vast space is given to spore forms of organisms and particularly to the anthrax agent (2, 5, 19).

In the opinion of foreign specialists, of special interest as possible agents of bacteriological warfare are those against which there are no effective means of specific prophylaxis and therapy, which will significantly hinder defense procedures. Thus, for example, Kliewe (4) has written: "As a bacteriological weapon there will primarily be used agents against which there are no or insufficiently effective means of immunization and treatment." Similar views have also been expressed by Phair (5) and other foreign experts. On the basis of the presented theory, special consideration has been given in the foreign literature in a contemplation of possible types of the bacteriological weapon to the agents of glanders and melioidosis, against which until the present time neither a vaccine nor sufficiently effective means of specific therapy has been developed.

Difficulty in the identification of the applied agents and also in the clinical and laboratory diagnosis of the illnesses caused by them acquire, according to the views of foreign authors, not a little significance in the selection of potential agents of bacteriological warfare. Serious handicaps in the early recognition of the illnesses do not prevent timely measures from being taken to combat them (2, 5). Some foreign authors consider that the best results can be rendered by agents of diseases not encountered or rarely encountered in a given locality and, consequently, are not known to a wide circle of doctors.

With the emergence of such diseases it is not possible to render the necessary means of specific prophylaxis.

therapy, and diagnostic methods. In addition, immunity is usually absent, or else there is a very low natural immunity, in man and animals to the infectious diseases exotic for the given locality (22). In virtue of these reasons there can arise serious difficulties in the recognition, prophylaxis, and treatment of similar diseases. For confirmation of this opinion it is customary to refer to the history of epidemic measles. In those localities where measles was never encountered and first appeared, it has always led to sizable epidemics among both children and adults.

However, not all foreign specialists share the opinion about the advantages of agents of infectious diseases which are exotic for a given locality. Repeatedly expressed views are that for purpose of the covert application of the bacteriological weapon agents of diseases will be used which are common or even endemic to the given locality, since the appearance of unusual, exotic illnesses will immediately provoke serious suspicion.

Briefly summarizing the comprehensive requirements discussed in the foreign literature for various agents of infectious diseases as agents of bacteriological warfare, the following basic points can be delineated:

- I. Pathogenicity of agent
  1. Virulence
  2. Infecting dose
- II. Brief characteristics of the induced illnesses
  1. Incubation period
  2. Severity
  3. Duration
  4. Fatality
  5. Invalidism
  6. Communicability
- III. Methods of cultivation of agents
- IV. Stability of agent
- V. Means of specific prophylaxis and therapy
- VI. Laboratory and clinical diagnosis

Later on this scheme will be used in a presentation of brief characteristics of individual agents of infectious diseases reported by foreign authors as possible agents of bacteriological warfare.

**BRIEF CHARACTERISTICS OF AGENTS OF INFECTIOUS DISEASES OF MAN AND ANIMALS WHICH, IN THE DATA OF THE FOREIGN LITERATURE, CAN BE USED AS AGENTS OF BACTERIOLOGICAL WARFARE**

At present there is extensive foreign literature devoted to proving the possibility of the use of various microorganisms as agents of bacteriological warfare.

First of all it is noted that agents, which completely satisfy all the requirements presented in the preceding section, do not exist. One microorganism, as, for example, the plague agent, is capable of inducing a severe illness with high fatality and communicability, but it has little resistance to influences of the external environment; others, like, for example, anthrax spores, display extreme resistance and produce a serious illness but they are not prone, however, to epidemic spread.

In view of such a situation various foreign authors prefer that each agent, depending first upon the objective, be considered for each specific case. It is certain that the greatest attention is attracted to the microorganisms which maximally satisfy the presented requirements.

Not stopping with the literature of the 20's and 30's, which now represents only historical interest, it is necessary to familiarize the readers with the basic considerations, published in foreign print of the last 15 - 17 years, on the possible use of individual agents as a bacteriological weapon. It seems to us that a formulation of the existing data of foreign literature in a table will help more graphically to demonstrate the significance of certain microorganisms as possible agents of the bacteriological weapon (Table 5).

Let us analyze the material presented in the Table according to groups of infections.

**Bacterial Infections.** In this group the greatest attention of foreign specialists is given to the agents of plague, anthrax, glanders, melioidosis, brucellosis, and tularemia. In connection with this in the following presentation the enumerated microorganisms and the diseases caused by them will receive a more complete description. Somewhat less attention will be given to the agents of cholera and icterohemorrhagic fever.

The agents of typhoid, paratyphoid A and B, bacterial dysentery, and food-borne salmonella in particular, are much less frequently mentioned as possible agents of the bacteriological weapon. Besides, many foreign authors generally consider the use of these agents for such purposes as remotely possible. Thus, for example, Fox (1)

concluded that agents of the intestinal group of infections are very doubtful in this situation.

Conducting a thorough and exhaustive study of the basic properties of the agents of infectious diseases, Rosebury and Kabat (2) have presented two lists of diseases; the first contains the names of diseases, the agents of which, in the opinion of these authors, can be used as possible agents of biological warfare, and the second list enumerates diseases whose agents have not been subjected to a study for these purposes. It is not without interest to note that the agents of typhoid, paratyphoid, salmonella, and even cholera were excluded by them in the second list.



Table 5a

## List

Diseases, agents of which have been named as possible agents  
of bacteriological warfare  
(according to data of foreign authors for period from 1942 - 1955)

Author (literary source)	1	2	3	4	5	6	7	8	9	10	11	12	13
Фокс, 1942	1	2	3	4	5	6	7	8	9	10	11	12	13
Английский институт микро- биологии, 1948	2	3	4	5	6	7	8	9	10	11	12	13	14
Роджерс и Келли, 1947	3	4	5	6	7	8	9	10	11	12	13	14	15
Апендикс, 1947	4	5	6	7	8	9	10	11	12	13	14	15	16
Купер, 1948	5	6	7	8	9	10	11	12	13	14	15	16	17
Уисман, 1948	6	7	8	9	10	11	12	13	14	15	16	17	18
Американский институт микро- биологии, 1948	7	8	9	10	11	12	13	14	15	16	17	18	19
Фарр, 1954	8	9	10	11	12	13	14	15	16	17	18	19	20
Кане, 1954	9	10	11	12	13	14	15	16	17	18	19	20	21
Балач, 1954	10	11	12	13	14	15	16	17	18	19	20	21	22
Крива, 1956	11	12	13	14	15	16	17	18	19	20	21	22	23
Берг и Крейг, с.м. 1956	12	13	14	15	16	17	18	19	20	21	22	23	24
Имя болезни	1	2	3	4	5	6	7	8	9	10	11	12	13
Чума (2)	+	+	+	+	+	+	+	+	+	+	+	+	+
Холера (2)	+	+	+	+	+	+	+	+	+	+	+	+	+
Сибирская язва (15)	+	+	+	+	+	+	+	+	+	+	+	+	+
Сибирская язва (15)	+	+	+	+	+	+	+	+	+	+	+	+	+
Сибирская язва (15)	+	+	+	+	+	+	+	+	+	+	+	+	+
Мелкошолос Бруцеллез (17)	+	+	+	+	+	+	+	+	+	+	+	+	+
Бруцеллез (17)	+	+	+	+	+	+	+	+	+	+	+	+	+
Туляремия (17)	+	+	+	+	+	+	+	+	+	+	+	+	+
Большой тиф и паратифы (19)	+	+	+	+	+	+	+	+	+	+	+	+	+
Бактериальная дизентерия (21)	+	+	+	+	+	+	+	+	+	+	+	+	+
Цереброспинальный менингит (22)	+	+	+	+	+	+	+	+	+	+	+	+	+
Церебро-геморрагический лептоспироз (болезнь Васильева-Вейля) (23)	+	+	+	+	+	+	+	+	+	+	+	+	+
Дифтерия (24)	+	+	+	+	+	+	+	+	+	+	+	+	+
Столбняк (25)	+	+	+	+	+	+	+	+	+	+	+	+	+
Газовая гангрена (26)	+	+	+	+	+	+	+	+	+	+	+	+	+
Пневмококковая пневмония (27)	+	+	+	+	+	+	+	+	+	+	+	+	+
Сальмонеллез (28)	+	+	+	+	+	+	+	+	+	+	+	+	+
Ботулизм (29)	+	+	+	+	+	+	+	+	+	+	+	+	+

KEY to Table 5a

- 1) Fox, 1942
- 2) English pharmaceutical journal, 1946
- 3) Rosebury and Kabat, 1947
- 4) Armagnac, 1947
- 5) Cooper, 1948
- 6) Wiesman, 1950
- 7) American magazine "Life", 1951
- 8) Phair, 1954
- 9) Cleve, 1954
- 10) Bullene, 1954
- 11) Creasy, 1955
- 12) Berger and Stevenson, 1955
- 13) Plague
- 14) Cholera
- 15) Anthrax
- 16) Glanders
- 17) Melioidosis
- 18) Brucellosis
- 19) Tularemia
- 20) Typhoid and paratyphoid fever
- 21) Bacillary dysentery
- 22) Cerebralspinal meningitis
- 23) Ictero-hemorrhagic leptospirosis  
(Vasil'yev-Weil's disease)
- 24) Diphtheria
- 25) Tetanus
- 26) Gas gangrene
- 27) Pneumococcus pneumonia
- 28) Salmonella
- 29) Botulism

Table 5b

3. Вирусные инфекции									
Грипп ①	+	+	+	+	+	++	++	+	++++
Пситтакоз ②									++++
Энцефаломелиты лошадей ③									++++
Лихорадка деңге ④									++++
Желтая лихорадка ⑤	-	-							+
Оспа ⑥							++		
Корь ⑦							++		
Свинка ⑧									
Лихорадка долины Рифт ⑨							+		+
Яшур ⑩									
4. Риккетсиозные инфекции									
Сыпной тиф ①	+								++++
Лихорадка Ку ②								+	
Лихорадка Скалистых гор ③									
Лихорадка цуцугамуши ④									
5. Протозойные инфекции									
Малария ①	-						+		++++
Трипаносомоз ②									
Токоплазмоз ③									
Амебная дизентерия ④								++	
6. Грибковые заболевания									
Кокцидиоз ①									++++
Гистоплазмоз ②									++++
Нокардиоз ③									++++
Бластомироз ④									++++

Условные обозначения: + может быть использован; ± использован; сомнительно; — использование маловероятно.

KEY to Table 5b

3. Viral infections

Grippe ①  
 Psittacosis ②  
 Equine encephalomyelitis ③  
 Dengue fever ④  
 Yellow fever ⑤  
 Smallpox ⑥  
 Measles ⑦  
 Mumps ⑧  
 Rift valley fever ⑨  
 Hoof and mouth disease ⑩

4. Rickettsial infections

Typhus ①  
 Q fever ②  
 Rocky Mountain spotted fever ③  
 Tsutsugamushi fever ④

5. Protozoal infections

Malaria ①  
 Trypanosomiasis ②  
 Toxoplasmosis ③  
 Amoebic dysentery ④

6. Fungous diseases

Coccidioidosis ①  
 Histoplasmosis ②  
 Nocardiosis ③  
 Blastomycosis ④

Symbols: + can be used; ± use doubtful; - use very doubtful

Although bacillary dysentery is also included in the first list, it is pointed out " that the agent of this disease presents very limited possibilities for its application for military purposes."

A similar point of view on the possible use of agents of intestinal infections for the bacteriological weapon have also been held by Armagnac (3) and a number of other foreign specialists.

Such opinions about the agents of intestinal infections are compiled in the foreign literature in respect to some of their characteristics.

The absence of optimal experimental evidence on these infections to some degree complicates a clarification of the likelihood of infection with various methods of application of the virus, particularly by the airborne route. In the opinion of a number of authors, airborne infection with intestinal infections is not very probable (1 and others).

In connection with this, even individual advocates of agents of intestinal infections as a bacteriological weapon indicate only the alimentary route of infection, particularly through water (6, 19).

A fact that acquires no little importance is that in the last 10 - 15 years the character of the clinical course of many intestinal infections has changed to quite an extent. Subclinical, atypical, and abortive forms, which proceed rather mildly, occupy a very large place. As for the salmonellas, they are always referred to as relatively mild illnesses which advance primarily as a type of food poisoning.

The presented data, so it seems to us, more or less explain the restraint, which foreign specialists show, in regard to agents of some intestinal infections in an evaluation of possible agents of bacteriological warfare.

Even more rarely mentioned than the agents of typhoid, paratyphoid, dysentery, and salmonella as possible agents of bacteriological warfare are those of wound infections - tetanus and gas gangrene - since there are effective ways and means of specific prophylaxis and therapy in these illnesses (2, 3).

In the order of elimination individual opinions are expressed about the possible use of agents of diphtheria, spinal meningitis, and pneumonia as a bacteriological weapon. However, most authors do not attach serious importance to these illnesses.

Thus, summarizing the material on the given group of diseases, it can be concluded that, in the opinion of

foreign authors, of the number of bacterial infections the most probable agents of the bacteriological weapon can be the ones of plague, anthrax, glanders, melioidosis, brucellosis, tularemia, cholera, and ictero-hemorrhagic leptospirosis.

In reference to this we must stop more in detail on the enumerated microorganisms and the diseases caused by them and give a brief character sketch from the point of view of the requirements made of possible agents of the bacteriological weapon.

**Toxins.** Of all the known toxins the one, which receives the most attention in the foreign literature as a possible agent of bacteriological warfare is the strongest poison of a biological nature - botulinus toxin (2), and it will be briefly characterized. Other toxins have not been considered in the literature as an agent of the bacteriological weapon.

**Viral Infections.** Most foreign specialists consider that of the viral infections the most probable agents of bacteriological warfare are the viruses of grippe, psittacosis, smallpox, equine encephalomyelitis, and dengue fever. Sometimes there are mentioned the viruses of yellow fever, measles, and hoof and mouth disease. Rosebury and Kabat (2) consider that "yellow fever answers all the requirements established for the selection of agents of bacteriological warfare." Besides dissemination by infected mosquitoes - vectors of the virus - the airborne route of infection is also possible in yellow fever, which imparts to this infection special importance (25).

The measles virus attracts some attention of the foreign specialists primarily because of the well-defined communicability of the disease (2, 22).

The same thing can be said also about the virus of infectious parotitis (mumps), which is mentioned as a possible agent of the bacteriological weapon even more rarely than the measles virus. The virus of hoof and mouth disease, in the opinion of Rosebury and Kabat (2), cannot be considered as a potential agent of bacteriological warfare against man, since the infecting activity of this virus is very low for man. Therefore, the virus of hoof and mouth disease, in their opinion, even if it can be used as a bacteriological weapon, it will only be against farm animals (large cattle, sheep, swine).

The virus of Rift valley fever, although put in the category of agents which can be used in bacteriological warfare by a number of authors (2, 5), nevertheless has not brought about serious arguments in respect to this.

Thus, from the presented data it is seen that of the group of viral infections the most emphasis is given by foreign authors to viruses of grippe, psittacosis, smallpox, equine encephalomyelitis, dengue fever, and yellow fever. We will elaborate later on the characteristics of the enumerated infections.

**Rickettsial Infections.** Of the number of infectious diseases of rickettsial etiology the foreign specialists consider the typhus agent the most likely one for the bacteriological weapon (2, 5). Moreover, in recent years separate communications have appeared in the literature verifying the possible use for these purposes of the agents of Rocky Mountain spotted fever, tsutsugamushi fever, and Q fever (5). Therefore, it is necessary to become familiar with this group of diseases in more detail.

**Protozoal Infections.** Of the protozoal diseases the agent of malaria is most often mentioned in the foreign literature. But it is noted that the use of the malaria plasmodium as an agent of the bacteriological weapon is coupled with extreme difficulties which stem from the complex method of derivation of the plasmodia in large numbers as well as by the limited possibilities of their application.

Most foreign authors consider that the only possible method of application of the malaria agent as the bacteriological weapon is by the spread of corresponding types of infected mosquitoes into areas with favorable conditions for their existence, multiplication, and transmission of the plasmodia to man. All of this places, in their opinion, under serious doubts the possibility of use of malaria plasmodia as a bacteriological weapon. For these same reasons, according to the viewpoints of foreign specialists, the use for military purposes of such protozoal infections as amoebic dysentery, trypanosomiasis, and toxoplasmosis is still less probable, although they are mentioned by individual authors as possible agents of the bacteriological weapon but without any basis. Therefore, there is no need to dwell in greater detail on separate representatives of this group of infections.

**Fungous Diseases.** In recent years in many foreign countries interest has risen considerably in agents of various fungous diseases, especially the deep mycoses, and this is evidently explained by the relatively severe clinical picture of these diseases as well as by the absence of developed measures of specific prophylaxis and therapy. Because of this we must go more into detail on the characteristics of the deep mycoses with the most severe course - coccidioidosis and nocardiosis.

The presented material demonstrate that from the large number of microorganisms which have been named by individual foreign authors as possible agents of bacteriological warfare, only about half merit more serious attention. Therefore, a short description will be given of the following agents and the diseases caused by them:

1. Bacterial infections: 1) plague, 2) anthrax, 3) glanders, 4) melioidosis, 5) brucellosis, 6) tularemia, 7) cholera, 8) ictero-hemorrhagic leptospirosis.

2. Toxins: botulinus.

3. Viral infections: 1) grippe, 2) psittacosis, 3) smallpox, 4) equine encephalomyelitis, 5) dengue fever, 6) yellow fever.

4. Rickettsial infections: 1) typhus, 2) Rocky Mountain spotted fever, 3) tsutsugamushi fever, 4) Q fever.

5. Fungous diseases: 1) coccidioidosis, 2) nocardiosis.

## BACTERIAL INFECTIONS

### Plague

Plague is an acute, highly contagious, infectious disease characterized by sudden intoxication, affliction of the cardiovascular system, inflammatory changes in the lymphatic glands, lungs, and other organs.

Pathogenicity of the agent. The plague agent has very high infectivity. It is assumed that a minimal infecting dose for man consists of a single microorganism (2). Man is highly susceptible to plague, and infection can occur through the respiratory tract, the gastro-intestinal tract, with a bite by infected fleas, and also with contact of the agent with the superficial skin and mucous membranes.

Symptoms of the disease. The incubation period ranges from several hours to six days and averages 2 - 3 days.

Plague can progress in five basic clinical forms: bubonic, pulmonary, septic, intestinal, and cutaneous. All the forms are characterized by a very severe course.

The disease begins usually with a severe headache, chills, rapid and high elevation of temperature; sometimes with an early impairment of consciousness, vomiting, and delirium. The speech of the patient is "slurred", and the gait is unsteady. The patient appears intoxicated. The skin of the face and conjunctive are injected, the



tongue is covered with a thick, grayish-white coating "furred tongue"). There is a distinct tachycardia. Later the liver becomes enlarged. The peripheral blood reveals a neutrophilic leukocytosis with an expressed degenerate-regenerate shift of the neutrophils. The disease advances with a serious condition of the patient and a high fever.

In the bubonic form severe pain is felt from the first day of illness at the site where a bubo eventually develops, on the 2nd day the bubo is already palpable, and on the 3 - 5th day it is completely developed. They are localized in the groin, over the axillae, on the neck and vary in size from a tiny induration to a lump the size of a large apple. The plague bubo is characterized by rapid development, sharp pain, pronounced periadenitis, rapid suppuration, and adherence to the skin and surrounding tissue.

In untreated cases fatality in bubonic plague ranges from 40 to 90% and averages 60%.

Frequently the bubonic form of plague is complicated by secondary plague pneumonia.

The primary pulmonary form of plague develops like a type of extremely severe hemorrhagic pneumonia with a rapid deterioration of the general condition of the patient and with a high fever (40 degrees or higher). An early impairment of consciousness is characteristic of the disease with manifestations of agitation, an increasing tachycardia and dyspnea, coughing with bloody sputum. There are often noted pleurisy pains, blood-tinged vomitus, and signs of sepsis (petechial rash, secondary bubos). The illness terminates with loss of consciousness, prostration, and coma followed by death.

In the absence of therapy the pulmonary form of plague terminates fatally in 3 - 6 days.

The septic form of plague is characterized by the absence of definite focal lesions, the presence of severe intoxication, and the development of numerous hemorrhages on the skin and mucous membranes. In the absence of specific therapy death follows on the 2 - 3rd day.

In the intestinal form, the existence of which is challenged by most authors, along with the general toxic manifestations there are frequently bloody, mucous stools. The illness usually terminates fatally.

The cutaneous form of plague is observed very infrequently.

In this form of the disease pock-like pustules or buboes with cloudy contents develop on the skin. Very often the cutaneous form changes to buboes.

The presented clinical characteristics of plague pertain to cases of the disease which are not treated with antibiotics or sulfanilamides. With treatment the clinical picture of plague, especially the pulmonary form, is significantly changed. Thus, in particular, in primary pulmonary plague there may be no bloody sputum, and the temperature can acquire an erratic character, etc.

Plague is one of the very contagious diseases. The pulmonary form is especially dangerous. Even a brief contact of a healthy person with a patient with the pulmonary form usually leads to illness.

Epidemics of plague in the absence of the necessary combative measures and prophylaxis results in a rapid spread.

Methods of cultivation of the agent. The plague bacilli grow rapidly on the usual nutrient media and can be obtained in large quantities.

Resistance of the agent. The plague agent does not have a pronounced resistance to the effects of various factors of the external environment. It is quickly killed under the influence of sunlight, high temperatures, and disinfecting agents. Elevated temperatures have an adverse effect on the preservation of the plague agent. A temperature of 55 degrees kills *Pasteurella pestis* in five minutes. With minus temperatures *P. pestis* is preserved much better.

Means of specific prophylaxis and therapy. Live vaccines prepared from avirulent EV and other strains are used for specific prophylaxis. A single subcutaneous, intracutaneous, or cutaneous vaccination is performed; a booster shot is given in a year. The live vaccine has been shown to give adequate protection experimentally to animals as well as epidemiologically when the bubonic form arises.

Due to some disadvantages of the live vaccines, American authors are continuing their search for a suitable chemical anti-plague vaccine.

In 1952, under the sponsorship of the epidemiological division of the United States Army at an official committee for the World Health Organization, a report of Meyer was given on the subject: "Latest Investigations on the Immunogenic Properties of Various Anti-Plague Vaccines."

The report specified that after special treatment of the dead plague cultures antigenic fractions could be recovered which had high immunogenic properties.

One of the fractions, called "fraction I", (representing a toxic protein substance soluble in water and ob-

tained from plague cultures treated with acetone) in a dosage of 0.1 mg safely protected mice from plague infection. Immunization of monkeys with "fraction I" protected 60% from subsequent infection with a massive dose of *P. pestis*.

In 1956 Silverman, Kyoshi Niguchi, and Ed. Meyer (26) reported on the recovery of an antigen from the plague organism on a synthetic nutrient medium containing pure amino acids and some other ingredients; this antigen had high immunogenic properties for white mice.

In 1955 Enrenkranz and Meyer (27) published a work in which they reported the results of the testing of three types of anti-plague vaccine on *Macacus rhesus* monkeys: a live one from the avirulent strain "EV-76", a formalized chemical vaccine from strain "Irek", and one called "fraction I".

The chemical vaccine was prepared from an extract recovered from plague bacteria of the strain "Irek", killed with acetone and lyophilized. The extract was precipitated and crystallized by Baker's technique.

The immunogenic properties of all the tested vaccines were about the same, but nevertheless a preference was shown for "fraction I". The authors considered that the immunogenic properties of all the vaccines tested by them depended on their content of "fraction I".

Efforts to further develop a chemical anti-plague vaccine have vigorously continued in the USA.

Streptomycin has been successfully administered as a means of specific plague therapy, and in the bubonic form of the disease it gives completely satisfactory results; treatment of the primary pulmonary and septic forms is effective only with the early (1 - 2nd day of illness) start of therapy.

A certain effect is obtained in the treatment of bubonic forms with sulfanilamides. Satisfactory results have been received with the administration of aureomycin, chloramphenicol, and terramycin, which are recommended in combination with streptomycin therapy. Penicillin is recommended to combat suppurative complications. Serum therapy at present has almost completely lost its value.

Laboratory and clinical diagnosis. The laboratory diagnosis of plague consists primarily of a smear examination and isolation of the agent.

A preliminary presumptive answer can be given on the basis of the smear examination of the sputum or the contents of the bubo in conjunction with the clinical and epidemiological findings.

The final diagnosis is reached in 2 - 3 days after the agent has been isolated by directly planting the sputum or bubo contents on a Petria dish and identified in pure culture. In isolated cases more time is required.

Considering the high communicability of plague, a battery of antiepidemic measures should be set up without waiting for the final bacteriological diagnosis, and they are governed by the results of the smear examination and the clinical and epidemiological findings.

In the clinical diagnosis of plague there is considerable difficulty in recognizing the primary pulmonary form which has to be differentiated from lobar and grippe pneumonia and also from the pulmonary forms of anthrax, tularemia, glanders, and psittacosis.

Therefore, in this situation it is necessary to give special attention to an analysis of the clinical-epidemiological and clinical-laboratory data, i.e., a combination of definite epidemiological, clinical, and laboratory findings which will help to establish the diagnosis

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Almost all the foreign authors, who have taken part in solving the problems of bacteriological warfare, consider that the plague agent best satisfies the requirements made of possible agents of bacteriological warfare; they believe that the application of *P. pestis* can be affected by two methods: through infected fleas and by the dispersion of pathogenic material from various ammunitions and devices.

Foreign authors are impressed by the strongly manifested retroactive quality of plague. Thus, for example, Rosebury and Kabat (2) consider that "the difficulty entails not how to cause the epidemic but how to cope with it if it starts." In the opinion of Fox (11) the use of the plague agent against troops on the battle field is scarcely probable because of the impossibility of protecting their own men from an epidemic. Therefore, the opinion exists (2) that the plague agent can be used at the fronts only for attack on completely isolated, fortified points; for example, naval or air bases which are located on islands. In view of the strong retroactive quality the plague agent is considered abroad as a weapon of strategic importance meant for the infection of the civilian population (1).

However, contradictory viewpoints also exist. Thus for example, the Kwantung Army Command in Manchuria intended to spread plague not only to infect the rear but also the front operations, and it was specifically proposed to spread plague-infected fleas upon the retreat of the Japanese Army.

## Anthrax

Anthrax is an acute infectious disease of zoonotic origin.

Pathogenicity of agent. The anthrax bacillus has very highly expressed infective activity in respect to man and many species of warmblooded animals. Rosebury and Kabat (2) consider that "only a few species of microorganisms surpass the anthrax agent in virulence for animals." Infection of man and animals can occur through the respiratory tract, the gastro-intestinal tract, and with contact of the agent on the superficial skin and mucous membranes.

In addition to man anthrax afflicts various species of animals. The most susceptible are sheep, goats, cows, horses, deer, buffalos, camels, swine, and many other carnivorous, wild animals.

Symptoms of the disease. The incubation period averages 2 - 3 days.

The disease is distinguished by an extremely severe course and high mortality.

Depending on the route of infection, three clinical forms of the disease are differentiated: pulmonary, intestinal, and cutaneous. A primary septic form is also observed.

The pulmonary form of anthrax proceeds like an extremely severe hemorrhagic pneumonia. With an onset of severe intoxication, high fever, and catarrhal manifestations affecting the conjunctivae and upper respiratory tract there develop dyspnea, cyanosis, and coughing with the discharge of watery, foamy sputum which is tinged with blood and then often coagulates, resembling jelly. The anthrax bacilli can be detected in the sputum. Pneumonia is usually accompanied by a dry and then an oozing pleurisy. After 2 - 3 days a large percent of the cases succumb with manifestations of collapse.

The intestinal form of anthrax is characterized by sharp pains in the stomach, bloody vomitus, and diarrhea accompanied by a highly acute anthrax sepsis. An ulcerative process develops in the intestines, usually in the

small intestine.

The disease is accompanied by high fever and severe intoxication. The patient expires after 3 - 4 days with manifestations of deep collapse.

The cutaneous form progresses less severely, but manifestations of intoxication are also pronounced in it. The length of its course is 2 - 3 weeks, and mortality reaches 15%.

Convalescence with anthrax is drawn out for 1 - 2 months.

Communicability of the cutaneous form is not high, but patients with the pulmonary form evidently can infect the community, liberating the agent with sputum.

Methods of cultivation of the agent. The anthrax agent multiplies rapidly on the usual nutrient media and can be obtained in large amounts.

Resistance of the agent. The characteristic property of the anthrax agent is the exceptionally high resistance of the spore to various influences of the external environment. They survive boiling in water for 30 minutes and dry heat with a temperature of 120 degrees for two hours. The spores are also highly resistant to the action of various disinfecting substances. In a 5% solution of phenol they are not killed for 40 days, in a 10% chloramine solution they survive for 10 hours, and in a 10% caustic soda solution and a 2% formalin solution for several hours. In the presence of organic substances and with lowered temperatures the sporicidal action of disinfecting substances becomes still less effective. Anthrax spores stay alive in the soil for ten years.

However, in spite of such high resistance of the anthrax spores there are at present effective means of decontamination for this disease including: methods of wet disinfection with the use of highly concentrated solutions (20%) of bleaching powder and chloramine, lye in a 2% solution of sodium carbonate for an hour, and also methods of chamber disinfection by steam and formaldehyde vapor with extended exposure.

Means of specific prophylaxis and therapy. Live vaccines prepared from virulent strains are used for specific prophylaxis of anthrax. With subcutaneous injection the live anti-anthrax vaccine creates a sufficiently potent immunity in animals.

In the USA chemical anti-anthrax vaccines are actively being developed for the immunization of people and agricultural animals.

In 1954 Wright, Narburg, and Slain (28) reported on

the recovery of an anthrax from cultures grown on a synthetic protein-free medium containing 17 amino acids and some other ingredients. The high efficacy of this vaccine was established with the testing of rabbits and monkeys: the animals usually survived after inoculation with 200 lethal doses.

In this same year Wright and associates conducted extensive experiments on *Macacus rhesus* monkeys, using an anthrax antigen precipitated with alum. A year after immunization with a two-stage vaccination with the alum antigen (0.5 and 1 ml subcutaneously) the monkeys survived intracutaneous injection of a dose of 50,000 - 100,000 virulent spores.

The monkeys, immunized twice subcutaneously with 1 ml of the alum antigen for each injection survived airborne infection with 890,000 - 3,000,000 virulent spores.

The anthrax antigen precipitated with alum was also tested on people (three-stage subcutaneously in a dosage of 0.5 - 0.5 - 0.5 ml); it proved harmless and slightly reactive.

Penicillin in combination with hyperemic blood serum is used in the treatment of the cutaneous form.

Excellent results have been received by the administration of large doses of penicillin and aureomycin. A positive effect has been obtained with terramycin and streptomycin. Serum therapy in the cutaneous form of anthrax has been discontinued because of the results of antibiotic treatment. There have been no conclusive findings on the efficacy of antibiotics in the pulmonary and intestinal forms. Treatment of anthrax meningitis (secondary) has proved unsuccessful with penicillin. A preliminary presumptive answer can be given after smear examination of the pathological material. The final diagnosis is reached 1 - 2 days after isolation of the culture.

Laboratory and clinical diagnosis. The laboratory diagnosis of anthrax is relatively simple.

The clinical diagnosis of the cutaneous form of anthrax does not present great complications, but the early recognition of the pulmonary and intestinal forms of the disease is extremely involved. The pulmonary form of anthrax has to be differentiated from the pulmonary form of plague, tularemia, glanders, psittacosis and grippe pneumonia.

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In conclusion it should be noted that in the foreign literature the anthrax agent has a foremost place as an agent of bacteriological warfare. Many foreign specialists consider that anthrax spores best satisfy all the requirements made of potential agents of the bacteriological weapon ( 4 and others).

Rosebury and Kabat (2) hypothesize that the anthrax agent can be used in bacteriological warfare by two basic methods: wounds - with the use of shell splinters, and air-borne - through infected air.

Wiesmann (19) et al do not exclude the possibility of using anthrax spores for alimentary and contact infection of people and farm animals.

Emphasis is placed on the high resistance of anthrax spores, which permits targets with an extended period of action and notably hampers decontamination (2).

The testimonials at the Khabarov trial (29) conclusively proved that the Japanese military criminals tried to use anthrax bacteria as a means of bacteriological warfare.

## Glanders

Glanders is a severe infectious disease of horses and other solid-hoofed animals; under natural conditions it is rather rarely encountered in man.

Pathogenicity of agent. The glanders agent is one of the group of microorganisms which has a pronounced infective activity in respect to man. Many cases of infection have been described in laboratories in spite of precautionary measures. Topley and Wilson (30) consider that this agent is one of the most dangerous organisms for laboratory workers. It is believed that in intralaboratory infections the airborne route is of primary importance.

Infection of man and animals can transpire through the respiratory tract, the gastro-intestinal tract, and mucous membranes.

Symptoms of the disease. The incubation period consists of 2 - 3 days, rarely more. In man glanders proceeds most often as an acute, septic disease with involvement of the skin, muscles, bones, and internal organs. The disease is characterized by great variations in the clinical picture. The most classical symptoms are a rash in the form of single blisters, ulcerative lesions of the nasopharynx, migrating pleuropneumonia accompanied



by coughing with bloody sputum, abscesses in the muscles, cones, and joints, and also debilitating dyarrhea. The onset of the disease is sudden, and the condition of the patients is very serious.

In the acute form of glanders fatality is 100%, and in the chronic form, which is observed in man very rarely, it reaches 50%.

The duration of the disease in the acute form is up to three weeks, in the chronic form up to a year or more. Glanders is very rarely transmitted from person to person although isolated contacts with the infection are dangerous.

Methods of cultivation of the agent. The glanders bacilli are easily cultivated on the usual nutrient media and can be obtained in large amounts.

Resistance of the agent. The glanders rod is one of the slightly resistant organisms. It tolerates elevated temperatures very poorly. With heating to 55 degrees it is killed in 10 minutes. It bears low temperatures relatively well. Dessicated cultures maintain viability for 3 - 4 weeks (2).

The glanders bacillus survives for a comparatively short time on objects of the external environment. In manure it dies in 12 - 15 days, in excretions from ulcers and the nasal mucous it dries up and dies after 7 - 15 days.

All of the common disinfecting substances quickly kill the glanders bacillus.

Means of specific prophylaxis and therapy. There are no vaccines for glanders. There are observations that animals, which have recovered from the disease, do not form a high degree of immunity, in view of which specific prophylaxis at present is a problem that is difficult to solve.

Specific methods of treating glanders have not been developed. In the USA six cases of acute glanders in man were treated with sulfadiazine (31). In addition, there data about the positive effect of serum therapy. However, the indicated means require broader approbation before a final decision is made.

Laboratory and clinical diagnosis. The laboratory diagnosis of glanders consists of the isolation of the agent by planting on glycerin agar or determination of the presence of the organism in the test material with the use of the Straus reaction in guinea pigs. These methods of laboratory diagnosis usually take 2 - 3 days. Chronic glanders in animals is diagnosed by means of the allergic

reaction with mallein (ophthalmic reaction).

The early clinical diagnosis of glanders presents certain difficulties. It is perplexing to differentiate acute glanders from the pulmonary forms of plague and anthrax. The clinical picture of glanders and melioidosis is especially confusing.

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The glanders agent more and more impresses the foreign specialists as a potential agent of bacteriological warfare, and this is explained by the fact that the glanders bacillus, from their point of view, quite adequately satisfies the requirement of these agents: this disease is characterized by a short incubation period and a very severe course with high mortality; there is no means of specific prophylaxis and therapy.

Rosebury and Kabat (2) and Wiesmann (19) consider that the most likely use of the glanders agent is in the form of an aerosol for airborne infection.

#### Melioidosis (pseudo-glanders)

Melioidosis is a rarely encountered, extremely progressive disease which is endemic for countries of South-eastern Asia.

Pathogenicity of the agent. The melioidosis bacillus has pronounced pathogenicity in respect to man and some rodents (wild rats and mice), which are the primary reservoirs of the agent in nature. Along with this there is described a spontaneous infection in cats, dogs, horses, and cows.

Infection of man and animals can occur through the respiratory tract, the gastro-intestinal tract, and mucous membranes. The melioidosis agent can penetrate into the body of man through injured integument. Vaucel (32) described three cases of melioidosis due to the contact of contaminated earth with a wound in an automobile accident.

Symptoms of the disease. The length of the incubation period in melioidosis has not been clearly established. Probably the authors are very nearly correct in saying it is several days (33).

The course of melioidosis in man most nearly resembles an acute, septic disease, which is characterized by a rapid and stormy clinical picture accompanied by fever (40 - 41 degrees), severe headache, tachycardia, dyspnea,

diarrhea and vomiting. Lobar pneumonia develops. By the end of the illness purulent pustules have often formed on the skin and abscesses in the muscles. The peripheral blood reveals a marked neutrophilic leukocytosis. Death ensues on the 2 - 5th day of illness.

Less often melioidosis occurs in the form of a subacute septic illness. This form is characterized by the development of suppurative processes in various organs (lungs, muscles, liver, spleen, kidneys, epididymis, etc). Subacute melioidosis lasts for 3 - 4 weeks and terminates in death.

Chronic melioidosis is encountered very rarely and is manifested by a chronic course of suppurative processes of various localizations. The illness is long drawn out (from several months to several years), bringing the patient to cachexis and death.

Person-to-person incidents of infection have not been described. The disease is considered noncontagious.

Methods of cultivation of the agent. The melioidosis agent is easily cultivated on the usual nutrient media.

Resistance of the agent. The melioidosis bacillus is distinguished by a relative resistance to the external environment. The organism survives in defecation and in the soil for not less than 27 days, and in drinking water for 44 days. Preserved well in the external environment under favorable temperature conditions, the agent quickly dies with elevated as well as with lowered temperatures. Heating of a microbial suspension to 56 degrees inactivates it even after a few minutes. The resistance of the organism is low also to the action of the common disinfecting solutions of bleaching powder, corrosive sublimate, etc. However, it is noteworthy that lysol and phenol have little effect (34).

Means of specific prophylaxis and therapy. There are no vaccines for melioidosis. Effective means of specific therapy have not been developed.

In recent years cases have been reported of the successful treatment of melioidosis with chloramphenicol and also with massive doses of sulfanilamide preparations in combination with penicillin therapy. Where the strains are not sensitive to chloromycetin, it is recommended that a combination be used: chloromycetin and aureomycin, or chloromycetin and terramycin (34, 35).

Laboratory and clinical diagnosis. Because of the variety of symptoms the clinical diagnosis of melioidosis is very complicated. In most of the cases in the literature it is not established while the patient is alive. In acute melioidosis a differential diagnosis has to be

considered with the pulmonary and septic forms of plague, acute glanders, tularemia, comatose malaria, and typhoid fever. The chronic form of melioidosis can simulate chronic glanders, brucellosis, tertiary syphilis, actinomycosis, nocardiosis, and several other illnesses.

The final diagnosis of melioidosis is established exclusively on the basis of laboratory tests which consist primarily of the isolation of the agent and its differentiation from the glanders bacillus.

The agglutination reaction and the complement fixation test do not have practical value. In some cases the Whitmore allergic test is resorted to (preparation similar to mallein).

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Foreign specialists have repeatedly indicated the agent of melioidosis as a potential agent of bacteriological warfare (2 and others) because they think that it, like the glanders bacillus, satisfies many of the prerequisites. Rosebury and Kaba\* (2) observe that the most probable use of the melioidosis agent is in the form of an aerosol for airborne infection.

## Brucellosis

Brucellosis is a generalized infectious-allergic disease occurring most often as a chronic sepsis with involvement of the locomotive system.

Pathogenicity of the agent. Brucellosis is caused in man by three forms of the agent: melitensis, abortus, and suis. Melitensis is the most pathogenic for man.

The agent of brucellosis has a very pronounced infective activity. Due to this, intralaboratory infections are encountered rather often in which the airborne route of infection apparently has primary significance (2). Meyer and Eddy (36), analyzing the data from 74 cases of intralaboratory illnesses, have shown that infection occurs through dust, sheep pens, and aerosol cultures forming from the centrifuge in operation.

The minimal infecting dose of the brucella agent is evidently very small for man, proof of which are the infections of individuals following single, brief visits to the laboratory (37).

Symptoms of the disease. Brucellosis is classified according to the severity of the course into the following forms: mild, moderately severe, and severe.

The incubation period averages from 2 - 3 weeks. The clinical picture of brucellosis is very variable and occurs in two basic clinical-pathogenic forms: acute septic and chronic migratory.

The acute septic form is marked by a prolonged course, undulating fever with remissions, enlarged liver, spleen, and lymph glands (micropolyadenitis) and is not accompanied by an expressed intoxication.

The chronic migratory form is differentiated from the acute septic and is characterized by migrating manifestations which appear primarily in the musculoskeletal, nervous, and reproductive systems. The illness can also start directly with migratory developments accompanied by a fever. The blood reveals a leukopenia, neutropenia, and a minor lymphocytosis. The duration of the illness ranges from several weeks to several years.

Involvement of the bones and joints is of special importance in the prognosis of occupational capabilities.

Mortality is not high in brucellosis and in the absence of chemotherapy ranges from 1 to 5%, and most fatal cases occur in the chronic form with a severe course involving the cardiovascular and nervous systems.

In the overall morbidity with brucellosis the mild and latent clinical forms are very important. The latent (asymptomatic) form comprises 13 to 25% of the entire morbidity rate.

The mild forms either generally do not disable man or disable for a very negligible interval of time.

At the present time there is no satisfactory evidence of the possible transmission of brucellosis from a patient to a healthy person. Therefore, the illness is practically considered noncontagious.

Methods of cultivation of the agent. All of the brucella agents are easily cultivated on enriched nutrient media and can be obtained in large quantities.

Resistance of the agent. The agents of brucellosis are capable of living on various objects of the external environment for a rather long time. Br. melitensis is preserved in the dried state for 60 - 80 days and survives in water for 30 days (28).

Disinfecting agents, direct sunlight, and high temperatures kill brucella. However, in comparison with other vegetative forms of organisms they have a rather pronounced resistance.

Means of specific prophylaxis and therapy. There are live vaccines prepared from avirulent strains of Br. abortus bovis and suis. The vaccines are intended for a single administration. It has been satisfactorily shown

in experiments that the immunity created by live vaccines is dependent both on the potency of the vaccine as well as on the duration of its action.

Projects on the preparation of chemical vaccines are being intensively developed in the USA.

The specific methods of treatment in brucellosis are considered to be vaccine and antibiotic therapy (aureomycin, chloramphenicol, streptomycin). The best results are obtained with a combination of both methods and the joint administration of several antibiotics.

Nevertheless the effectiveness of the treatment of brucellosis, especially of the chronic cases, must be considered inadequate because it does not guarantee that relapses will not occur.

Laboratory and clinical diagnosis. In view of the great variety of symptoms and the presence of different forms of the disease, the clinical diagnosis presents serious difficulties. Therefore, the final diagnosis can be established from the use of laboratory methods. The agglutination reactions have the most practical significance (Wright and Huddleston reactions) and the allergic intradermal test (Burn reaction). More infrequently determinations of the opsonic-phagocytic index are used.

It must be kept in mind that vaccinated persons can also have positive immunological reactions.

Isolation of the agent from the patients involves certain problems and requires considerable time and special procedures in the laboratory.

The agent of brucellosis has been given an essential status abroad as a possible agent of the bacteriological weapon. The airborne route of infection attracts the greatest attention.

Discussing the question of the possible use of the brucella agent as a bacteriological weapon, Rosebury and Kabat (2) wrote: "The assumption cited in the preceding sections (indicating the possibility of airborne infection by the culture) may appear decisive in the possible use of brucella as a bacteriological weapon. It must also be acknowledged that all the other aspects indicate brucella of the melitensis type as one of the most promising implements that can be used for this purpose." Phair (5), Creasy (21), and others arrived at the same conclusions. Notwithstanding, some foreign authors, commenting on the low mortality rate with brucellosis, consider this fact a negative quality of the agent as a type of bacteriological weapon. However, along with this they state that "attacking optionally should not always try for the annihilation of people; it can make them sick. And this will be enough

to deprive people both of military efficiency and working ability. This also increases the man-hours and overflows the hospitals." To fulfill this task the agents of brucellosis were named as possible types of a bacteriological weapon (20, 21).

### Tularemia

Tularemia is an acute disease of a septicemic character with common involvement of the lymphatic system.

Pathogenicity of the agent. The tularemia agent has highly expressed infectivity in respect to man and some kinds of rodents (mice, water rats, etc). Just as with brucella, the work with tularensis organisms often leads to intralaboratory infections. Rosebury and Kabat (2) consider that the "agent of tularemia is one of the most infectious for man."

Infection in man occurs through the respiratory tract, the mucous membranes of the eyes, and the digestive tract. The organism can penetrate through the injured skin.

There are no data given in the literature on the size of the minimal infecting doses for man. However, on the basis of an analysis of described cases of intralaboratory infections it must be assumed that, as for brucellosis, these doses are very small.

Symptoms of the disease. The incubation period averages 3 - 7 days and is sometimes shortened to several hours. Four basic forms of the disease are distinguished: bubonic, abdominal, generalized (typhoidal), and pulmonary. Regardless of what form it will take, tularemia in man begins suddenly and acutely.

There is marked elevation of temperature, and clearly expressed manifestations of intoxication develop. The patient complains of head and muscular pains; face, conjunctivae, and throat become hyperemic.

The bubonic and ulcerative forms of tularemia are marked by the development on the 2 - 3rd day of illness of mild soreness at the site of the future bubo, from the 3 - 6th day the bubo becomes palpable, and on the 6 - 7th day it reaches full development. Tularemia bubos differ from plague ones by slower development, mild tenderness, absence of skin changes over them, and periadenitis. The bubo ranges in size from a walnut to a chicken egg. Infection occurs through the skin (including bites) with the possible development of ulcers in the regions of the original portals of infection.

Clinical variants of the bubonic form of tularemia are the eye bubo and the tonsillar bubonic form.

The abdominal form of tularemia is marked by severe pains in the stomach accompanied by high fever and manifestations of intoxication. Inflammatory changes in the mesenteric lymphatic glands are the primary site of the illness. Infection occurs through the alimentary route.

The generalized (typhoidal) form differs by the development of severe manifestations of intoxication and high fever which appear in the absence of any other changes.

The febrile period lasts for several weeks (usually about three weeks) and is accompanied by varying kinds of skin eruptions (erythematous, hemorrhagic). The development of secondary bubos is possible.

The pulmonary or bronchopneumonia form of tularemia, arising as a result of inhaled infection, has its course in pneumonic and bronchial variants.

In the pneumonic variant tularemia pneumonia develops which is characterized by a weak and prolonged course, tendency for suppuration (abscesses, gangrene), and also by late percussion and auscultatory manifestations.

In Europe tularemia mortality does not exceed 0.5 - 1%; in the United States of America it is somewhat higher and reaches 4 - 5 and even 7%.

Tularemia is not transmitted from person to person, so it belongs to the group of noncontagious diseases.

Methods of cultivation of the agent. Cultivation of the tularemia organism presents certain difficulties since it does not grow on simple nutrient media. The tularemia agent is usually grown for 3 - 4 days on blood agar containing glucose and cystine.

Thus, to obtain cultures of the tularemia agent in large quantities is somewhat complicated and presents more of a problem than procurement of a culture of agents of anthrax, plague, glanders, melioidosis, and brucella.

Resistance of the agent. The tularemia agent displays considerable resistance to the effect of various factors of the external environment. With a temperature of 60 degrees it is killed after only 5 - 10 minutes. The organism has very little sensitivity to the action of low temperatures, and in frozen meat it can be preserved for three months.

French (63) established that tularensis bacteria maintain their virulence if they subsist in the organs of infected animals or are immersed in undiluted glycerin (a virus tool); for a month at room temperature (18 - 20 degrees), for six months at 10 degrees, and for 10 years or more at a temperature of -14 degrees.

The tularemia organism displays a high degree of resistance to dessication. Thus, in skins removed from



rodents sick with tularemia and stored at room temperature, it is possible to maintain their viability for 1 1/2 months.

Disinfecting substances kill the tularensis organism rather quickly: 1% solution of cresol in two minutes, 0.1% formalin solution after 24 hours (2).

Means of specific prophylaxis and therapy. There is a live vaccine for a single cutaneous injection. In the USA a chemical vaccine has been developed with a two-three-stage inoculation. The effectiveness of immunization is satisfactory.

Treatment with antibiotics gives excellent results: streptomycin, chloromycetin, aureomycin, and terramycin.

Laboratory and clinical diagnosis. The clinical symptoms of tularemia are very varied. In the diagnostic workup of the disease a differential diagnosis from plague, anthrax, typhoid fever, and brucellosis has to be made. Especially important is the early differential diagnosis of tularemia from the bubonic form of plague. Incidents of tularemia with bubo development has repeatedly resulted abroad in confusing it with the bubonic form of plague. It is imperative to distinguish tularemia pneumonia from plague and anthrax pneumonia as soon as possible, and this is very difficult, especially in the first days of illness. In connection with this it is very perplexing to establish a diagnosis of tularemia only on the basis of clinical signs.

The laboratory methods of diagnosis are of decisive importance. Isolation of the agent from patients is involved and requires considerable time (5 - 7 days).

The intradermal allergic test with tularin is of the greatest practical value in the diagnosis of tularemia. It is possible to establish a diagnosis of tularemia in man by this reaction in 3 - 6 days from the onset of the illness. The agglutination reaction is also used along with the intradermal test. For practical purposes the slide agglutination test with a drop of blood is the most convenient.

It must not be forgotten, however, that the immunological reactions and the tularin skin test can be positive in vaccinated persons.

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Almost all of the foreign specialists, who confidently avow in print the possible use of individual types of microorganisms as agents of bacteriological warfare, consider that the agent of tularemia is completely accep-

table for these purposes.

Rosebury and Kabat (2) very plainly declare that "the agent of tularemia can be recommended to those who seek a potential means for bacteriological warfare". Similar opinions have also been expressed by Phair (5) and others.

In the foreign literature the tularensis agent is given approximately the same importance as the brucella agent as a potential type of bacteriological weapon. Bul-lene (20) and Creasy (21) think that the tularensis organism can be used where it is essential to induce mass sickness on people and not result in death.

There are also individual comments in the foreign literature on feasible methods of applying the tularensis organism for military purposes, and the airborne route of infection receives the most emphasis. Thus, for example, Rosebury and Kabat (2) wrote: The airborne route of dissemination of the tularensis agent is the most appropriate for bacteriological warfare."

## Cholera

Cholera is an acute infectious disease of man, prone to a rapid epidemic spread and characterized by high mortality.

Pathogenicity of the agent. Marked polymorphism and mutation is innate to the cholera vibrio, and along with variations in morphological, cultural-biochemical, and serological properties the virulence of strains also varies within wide ranges, down to a complete loss of pathogenicity for man. Mutation of the cholera vibrio in cultivation under laboratory conditions is especially widely manifested.

The dosages are not indicated in the literature which are necessary for the infection of man.

Infection occurs chiefly through the mouth. Entering the body, the agent multiplies in the small intestine. Other routes of possible infection have not yet been proved in man.

Symptoms of the disease. The incubation period averages 2 - 3 days but it can range from 1 to 7 days.

The clinical picture of cholera varies widely from ambulatory to the most severe form of the disease. In general, five basic forms are observed: cholera dysentery (cholera enteritis, diarrhea), cholera, algid cholera, "fulminating" cholera, "dry" cholera. The latter two are rarely encountered.

Cholera dysentery (cholera diarrhea) is marked by

manifestations of a mild enteritis with soft or liquid stools 4 - 10 times in a day. In some patients the disease may change to the next stage.

Cholera (or, more precisely, cholera gastroenteritis) has a distinctive syndrome: vomiting, diarrhea, and cramps. The condition and state of health of the patient is serious.

The next, still more severe form of the illness is the algid which is characterized by hypothermia, dehydration, and collapse. In this stage a fatal outcome can result.

"Dry" cholera (cholera sicca) proceeds without diarrhea and vomiting, and the patient expires from intoxication even before the development of enteritis symptoms.

"Fulminating" cholera terminates in death a few hours after the onset of the disease.

The duration of the illness in the usual course is from two days to two weeks. Complicated by cholera typhoid, recovery is delayed, and the mortality rate rises. The average mortality is about 50% in cholera. Patients with all forms of cholera are contagious from the first day of illness through the entire course. Because of this they must be kept in isolation with special antiepidemic regime.

In 1 - 2% of the recovered cases a carrier state is observed for one or, rarely, three months.

Cholera is included in the diseases which are marked by highly expressed contagiousness, which explains its rapid epidemic spread.

Methods of cultivation of the agent. The cholera vibrio is easily cultivated on the usual nutrient media and can be obtained in large quantities. However, during cultivation the agent often undergoes considerable mutation, including a change in pathogenic properties down to a complete loss of pathogenicity.

Resistance of the agent. Outside the body of man the cholera vibrio displays little resistance to various factors of the external environment. Direct sunlight kills the vibrio in several hours. They have very little resistance to drying and high temperatures. With a temperature of 100 degrees the cholera vibrios are killed instantly, with 80 degrees after five minutes, and at 56 degrees after 25 to 30 minutes.

Many disinfecting agents possess high bactericidal action against the cholera vibrio. Corrosive sublimate in a dilution of 1:1000 kills the vibrios in 15 seconds, 1% phenol after five minutes.

It should be mentioned that low temperatures show

a noticeable protective influence on the maintainance of the cholera vibrios.

There is extensive literature devoted to the preservation of cholera vibrios in patients' feces, linen, soil, water, and food products. The preservation of the cholera vibrios in these objects extends from several hours to several months.

These conflicting findings on the preservation of cholera vibrios is probably explained by different temperatures of the environment, the reaction of the medium, the chemical constituency of the substrate on which the vibrios were maintained in the investigations, and also the quantitative and qualitative extraneous microflora which exhibited an antagonistic effect on them.

Means of specific prophylaxis and therapy. There are bacterial vaccines (heated or formalized) and also vaccines prepared from "complete antigens". The effectiveness of these preparations is approximately the same as that of the typhoid-paratyphoid vaccines. Oral immunization with tablet vaccines has little effect. Bacteriophages are also employed as a means of specific prophylaxis.

The earlier treatment is started, the more effective it is. A cholera bacteriophage is widely used in therapy which gives excellent results at the onset of the illness and in mild forms (enteritis). For the treatment of cholera and the algid form an intravenous infusion of hypertonic solutions, plasma, or blood are used, and symptomatic agents are also administered. The application of heat is very important. The effect of antibiotics and sulfanilamides is still insufficiently studied. There is evidence of the value of the application of the poorly soluble sulfanilamide preparations of the sulfaguanidine type and also of terramycin, which aids in vibrio excretion.

Laboratory and clinical diagnosis. In typical cases the clinical diagnosis of cholera does not present any difficulty since the symptom complex of cholera gastroenteritis, algid, and typhoid is defined clearly enough. In some cases a differential diagnosis has to be made from food poisoning infections, different poisons (toxins of mushrooms, ticks, arsenic, methyl alcohol, corrosive sublimate, etc), and cholera-like forms of malaria.

Results of the bacteriological investigation are decisive in the diagnosis. It has to be kept in mind that every case of illness suspected of being cholera should be examined bacteriologically in the most careful manner without regard as to whether the clinical picture corresponds to these findings. A preliminary bacteriological diagno-

sis can be established on the basis of the smear examination of the pathological material in several minutes and by the culture method in 8 - 10 hours. However, the results of the agglutination reaction, which helps to distinguish the unknown organism from the cholera agent, is conclusive in the differentiation of true cholera vibrios from cholera-like forms. Therefore, a definite answer can be obtained from the laboratory in 2 - 3 days.

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In a consideration of aspects of bacteriological warfare in the foreign literature of the 1920's and 1930's the most emphasis was placed on agents of intestinal infections, particularly cholera. However, in the following years the viewpoints on the possible use of these agents for warfare purposes have changed. Fox (1), Rosebury and Kabat (2), Armagnac (3), and other foreign authors already in the 1940's were expressing very definite opinions about the low potentiality of the cholera vibrio as an agent of bacteriological warfare.

In the foreign literature of recent years the cholera vibrio has again been mentioned as a possible type of bacteriological weapon (6, 19). However, no facts have been offered to substantiate the expressed points of view.

The evidence of the Khabarov trials (29) convincingly demonstrates that the cholera vibrio was not ignored by the armies of the capitalistic states as a possible agent of bacteriological warfare.

#### Ictero-hemorrhagic leptospirosis

Leptospiral jaundice is an acute infectious disease characterized by intermittent fever, hemorrhagic and liver-kidney syndromes.

Pathogenicity of the agent. The agent of the disease apparently has high infectivity for man and also for rats - the source of leptospira under natural conditions.

Numerous incidents of sickness in laboratories prove the marked infectiousness of leptospira.

The infection in man occurs through the mucous membranes of the mouth, respiratory tract, eyes, and gastrointestinal tract.

Symptoms of the disease. The incubation period lasts for 8 - 15 days. The onset of the illness is sudden with chills and fever up to 39 to 40 degrees. From the

very first day the general condition becomes serious and consciousness dimmed. An intermittent fever and capillary hemorrhages on the conjunctivae, the skin of the chest and the extremities are characteristic. On the 5 - 6th day of illness jaundice appears, the liver is tender and extends beyond the costal rib. Very often the kidneys show involvement, which is manifested in nephritis.

The illness usually lasts for 3 - 3 1/2 weeks, sometimes up to 1 1/2 months. The prognosis is largely determined by the age of the patient; the mortality rate is especially high among patients older than 50. Fatality varies in different countries in rather broad ranges: in Japan 16.9 - 29.9%, in Holland 8.5%, in England 15%, in the USA 30%, in Sweden 15%.

In spite of the fact that patients with leptospiral jaundice excrete the agent in the urine, there is no record in the literature of illness arising as a result of infection from a patient. Therefore, leptospiral jaundice has to be considered practically as a non-contact disease.

Methods of cultivation of the agent. The cultivation of leptospira is not much of a problem. Leptospira are not fastidious concerning the composition of the nutrient media and are successfully grown on common tap water with an additive of a trifling amount of protein (5 - 10% blood serum). Noguchi (64) proposed a method of mass cultivation of leptospira in large flasks of semisolid medium 1 - 2 cm deep. He reported that after 2 - 3 weeks the concentration of leptospira came up to 16 milliard in 1 cm<sup>3</sup> of medium. Leptospira are also successfully propagated in the chorioallantoic membrane of chick embryos.

Resistance of the agent. Leptospira die quickly with heating and under the influence of disinfecting solutions. They are especially sensitive to even insignificant concentrations of acids and salts.

With heating to 50 degrees the leptospira die in 25 - 30 minutes. Direct sunlight kills during the first hour of exposure. Drying also causes their rapid death. Active chlorine in a dose of 2 mg/liter kills leptospira in several hours, a 0.5% phenol solution in 20 minutes.

At the same time leptospira tolerate freezing well. Even prolonged freezing with subsequent thawing does not produce death.

Leptospira are capable of surviving for a long time in water in natural basins. Experimental data and epidemiological observations have established that this period is 3 - 4 weeks.

Means of specific prophylaxis and therapy. Specific prophylaxis is accomplished by vaccination. Two vac-

cines are known: a killed one and one prepared from attenuated leptospira.

Penicillin and specific leptospira anti-serum are used with success for treatment. Therapy is especially effective if it is started before the fifth day of illness.

Laboratory and clinical diagnosis. The clinical diagnosis of leptospiral jaundice is not very difficult. However, in some instances a differential diagnosis between it and jaundice of other etiologies. It must be kept in mind that sometimes ictero-hemorrhagic leptospirosis can occur without jaundice.

The laboratory diagnosis is made on the basis of a blood culture (before the 5 - 6th day of illness) and a urine culture (after the 12 - 15th day of illness). Growth of the leptospira starts between the 7th and 10th day but it may be considerably delayed. Therefore, for practical purposes the culture method is of little use. The most acceptable is the direct microscopy of blood and urine; this method is very simple and easily done.

Starting with the 6 - 8th day of illness the blood sera of patients reveal agglutins, lysins, and complement fixing antibodies which can be determined by the proper immunological reactions.

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During the 1947 - 1955 period the question about the possible use of the agent of leptospiral jaundice as an instrument of bacteriological warfare was repeatedly discussed in the foreign literature (2, 5, 6). Actually, the cited authors did not argue the point but merely confined themselves to accepting this microorganism on the list of potential agents of bacteriological warfare. Rosebury and Kabat (2) undertook experiments to substantiate their point of view. However, analyzing the possible mechanisms of infection of man, they decided that "the use of this agent as a means of attack would of necessity be limited to the contamination of food and especially of water." Nevertheless, in the chapters of their monograph devoted to the possible use of agents of intestinal infections for these purposes, these same authors were in doubt about the effectiveness of the water factor in bacteriological warfare.

## TOXINS

### Botulism

Botulism is a severe intoxication caused by bacterial toxins of the botulinus microorganism.

Pathogenicity of the agent. The botulinus toxin is apparently the most potent biological poison for man. The minimal lethal dose of the botulinus toxin is trivial in amount for man. Thus, Dixon (40) reported case histories where death was caused merely by tasting contaminated food without swallowing an appreciable amount of it. Data on many outbreaks of botulism in man indicate that with a rare exception all of the people who partook of the food containing the toxin became ill. This fact convinces us that the percentage rate of poisoning is almost 100.

Summer (41) determined the minimal lethal dose of the toxin for white mice with subcutaneous injection of 0.0000002 gram per one kg body weight of the animal. Rosebury and Kabat (2), assuming a similar sensitivity of man and mice to the toxin, consider that the minimal lethal dose of the toxin for man can be set at 0.00002 gram. Ducca and Wood (14) found that the lethal dose of the toxin for *Macacus rhesus* monkeys with oral introduction was lower than the weight equivalent of the minimal lethal dose for mice with intraperitoneal administration. Therefore, Rosebury and Kabat suggested that the actual minimal lethal dose for man with oral introduction was perhaps not far from the cited value.

Botulism occurs as a result of the entry of the toxin into the digestive tract. An equally potential method of infection is also the airborne route (2).

Symptoms of the disease. The incubation period is short - from several hours to 2 - 3 days. The length of the incubation period usually is an indicator of the severity of the illness. The illness develops quickly. At first there are signs of extreme weakness, depression, and loss of appetite. Sometimes there is headache, pain in the stomach, nausea and vomiting, increased salivation changing to dryness in the mouth. A specific diagnostic sign is the so-called symptomology: blurred vision, diplopia, absence of reaction to light, mydriasis, ptosis, strabismus, and ophthalmoplegia. Bulbar manifestations develop after the "eye symptoms": dysphagia, disphonia, and respiratory disturbance. The temperature usually (in the absence of complications) is normal or subnormal. The severity of the patient's condition rapidly increases, and in fatal cases death sets in from paralysis of the



respiratory center with complete maintainance of consciousness. Patients, who survive the first 10 days, usually recover. However, convalescence proceeds slowly for weeks or even months.

The occupational capacity of those who suffer the illness remains sharply curtailed for some months. Rapid exhaustion, dyspnea, and palpitations result from even the most insignificant physical exertions.

Mortality is high in botulism; during some outbreaks it has reached 60 - 85%.

The illness is noncontagious.

Methods of cultivation. Recovery of the botulinus toxin even in large quantities, in the opinion of foreign specialists, does not present serious difficulties. As long ago as the 1940's Summer (40) developed a technique of preparing relatively large amounts of active botulinus toxin. Very simple facilities and reagents are needed for this technique.

Resistance. The botulinus toxin exhibits some degree of resistance to the influence of various factors of the external environment. It tolerates heating fairly well and is destroyed at a temperature of 73 degrees after 10 minutes, at 80 degrees after six minutes.

The toxin can be preserved at room temperature for several months. It is extremely resistant to cold and to freezing with subsequent thawing (2).

Means of specific prophylaxis and therapy. Botulinus anatoxins can be employed for the specific prophylaxis of botulism.

It is recommended that botulinus anti-serum be used as a basic means of special prophylaxis.

Therapeutic botulinus anti-serum is also administered for the treatment of poisoning by the botulinus toxin. Serum therapy in the early stages is an effective method of treatment but it does not help appreciably in reducing fatality. However, a later start of treatment (two days or more) makes this method of therapy almost useless.

Clinical and laboratory diagnosis. The symptom complex of botulism in classic cases is so characteristic that the establishment of the diagnosis does not comprise great difficulty, but mild and masked cases are very complicated to recognize. In the differential diagnosis it is primarily necessary to exclude food poisoning infections, poisoning (atromine, methyl alcohol, mushrooms) and encephalitis.

Proof of the clinical diagnosis is the finding of

the toxin in the stomach contents, gastric lavage, blood of patients, and also at autopsy. The toxin may be detected in food products, water, and other things as well.

The most rapid method of identification of the toxin in material to be tested is the injection of white mice with the biological specimen.

The laboratory diagnosis usually takes 1 - 2 days although a preliminary result can be obtained in several hours after the appearance of specific symptoms in the infected white mice.

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Considering the problem of using the botulinus toxin in bacteriological warfare, many foreign specialists rate it very highly in this respect (1, 19, and others). In defending their viewpoints they start first with the extremely high infective property of the botulinus toxin for man.

The question about the possible use of the toxin of botulism as an agent of bacteriological warfare has been open to discussion in the foreign literature; at the same time some concrete ideas about probable methods of its application have been expressed. Thus, Rosebury and Kabat (2) consider that the botulinus toxin can be used for purposes of bacteriological warfare by the following methods: dispersion from a plane to contaminate various water basins with drinking water and dissemination of a dry powder toxin through the air. The idea has also been projected about the potential use of the botulinus bacilli to contaminate food products, especially canned goods in canned goods factories, and this is a method for saboteurs. However, Rosebury and Kabat think this method unlikely.

## VIRAL INFECTIONS

### Grippe

Grippe is an acute infectious illness of man, prone to rapid epidemic spread with a shift under certain conditions to a pandemic.

Pathogenicity of the agent. The grippe virus has high infectivity for man. Minimal infecting doses for people apparently are very small, which indicate frequent infections even after brief contact with patients. Only

the viruses found in drops of mucous, saliva, and sputum of patients are dangerous for man. The grippe virus, isolated from a patient and adapted to the chick embryo or laboratory animals, at first partially loses its pathogenicity for man and then completely.

Symptoms of the disease. The incubation period is brief and often consists of 1 - 2, rarely 3 - 4 days. The onset is acute: chills, headache, general malaise and feeling of breakdown, temperature elevation to 39 0 40 degrees. Some cases develop catarrhal manifestations, head cold, and coughing. Pains in the superciliary arch are typical and a feeling of stuffiness in the chest (tracheitis). The temperature remains elevated for 3 - 5 days after which it returns to normal, and recovery sets in. Weakness and general malaise continue for several days after recovery from the illness.

Grippe can occur in a severe form with the development of hemorrhagic pneumonia often resulting in death. In addition, during an epidemic there are often mild, debilitating, and asymptomatic forms.

Grippe is one of the group of highly contagious illnesses transmitted by the airborne route. Epidemic grippe can take on extensive proportions after a fairly short time and in some places envelop more than 40% of the population. Periodically a huge epidemic springs up which sometimes acquires a pandemic distribution.

Patients must be isolated for effective prophylaxis, which, however, is difficult to manage in mass sickness.

Methods of cultivation of the agent. The grippe virus is well cultivated on fertilized chick embryos with inoculation of the amniotic or allantoic sac, in the lung tissue of mice with their intranasal infection, and also in tissue cultures.

Cultures of the virus obtained by the enumerated methods, as has already been indicated above, appreciably and sometimes completely lose pathogenicity for man.

Resistance of the agent. The grippe virus displays little resistance to various factors of the external environment. In a 5% suspension of mouse lung the virus is inactivated at a temperature of 60 degrees in 30 minutes. The virus is preserved at refrigerator temperature in glycerin for three months, at a temperature of -20 degrees for many months.

Sunlight rapidly inactivates the grippe virus. It tolerates drying in the air poorly. The sprayed virus is quickly inactivated. However, drying in the frozen state (lyophilization) does not destroy the virus.

At the present time there has been shown a high sensitivity of the grippe virus to the application in practice of disinfecting agents: phenol, corrosive sublimate, bleaching lime, and chloramine, etc.

Means of specific prophylaxis and therapy. Active immunization is performed with anti-grippe vaccines. The most widely used at present is the live vaccine prepared from a strain of low virulence. The vaccine is introduced into the nasal orifice by means of drops or a spray. According to preliminary data, morbidity in an epidemic period is successfully reduced about 1 1/2 times by vaccination.

As prophylactic agents during a grippe outbreak there is used an anti-grippe serum with insufflation, and also ecmolin, acrichine (quinacrine), et alia. The efficacy of these agents is not high.

Specific anti-grippe sera are used to treat grippe, and antibiotics are administered to combat secondary infections (terramycin, penicillin, and ecmolin) as well as chemotherapeutic agents.

Laboratory and clinical diagnosis. In an epidemic period the establishment of a clinical diagnosis of viral grippe does not constitute a great difficulty. In between epidemic periods the diagnosis of grippe is not easy, and it is necessary to perform a differential diagnosis with acute catarrhs of the upper respiratory tract produced by adenoviruses, typhoid fever, Q fever, psittacosis, water fever, pappataci fever, and other illnesses.

Of the diagnostic laboratory methods for grippe emphasis is placed on the hemagglutination-inhibition reaction and on routine blood tests. However, the methods of specific diagnosis of the illness during the early period of its development have still not been developed.

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Numerous foreign specialists are in favor of the possible use of the grippe virus for purposes of bacteriological warfare (5, 6, 22, etc).

Rosebury and Kabat (2) consider that the grippe virus can be used independently as well as in combination with other bacterial and viral agents. However, they additionally note that "the suitability of a virus as an agent of bacteriological warfare and the military use of this virus are questions which can only be satisfactorily resolved after further study."

## Psittacosis

Psittacosis is an acute infectious illness of man and birds, occurring in man with specific involvement of the lungs.

Pathogenicity of the agent. The infectivity of the virus is very high for man, as a large number of intralaboratory infections indicate. Infection takes place in the work with infected birds as well as with virus cultures. Much interest has been shown in the incidence of sickness among laboratory workers not directly engaged in the viral study but only visiting the room where the work was being performed (2).

Infection of man occurs by the air-droplet and air-dust routes through the respiratory organs.

Symptoms of the disease. The incubation period in psittacosis lasts from 7 to 14 days and averages 10 days. The onset usually is acute and sudden, and the temperature rapidly becomes elevated (39.0-40 degrees), and a specific psittacosis pneumonia develops.

The duration of the illness is 2 - 3 weeks, and sometimes even longer. Convalescence often progresses very slowly and frequently is interrupted by relapses.

Before the administration of antibiotics fatality with psittacosis ranged from 11 to 20% and sometimes reached 30%. Since the introduction into medical practice of antibiotic therapy the prognosis in this disease has become more favorable. With a timely start and the correct conduction of therapy mortality in psittacosis is reduced to 2%.

Psittacosis belongs to the group of contagious diseases. Infection is possible by contact with the patient during the febrile phase of sickness, especially when coughing takes place. Therefore, isolation facilities should be devised for the recovery of patients with psittacosis with a consideration of a special antiepidemic routine. However, it should be stressed that human psittacosis is considerably less contagious than psittacosis in birds, and transmission of the illness from person to person, even in caring for the patients, transpires rather rarely.

Methods of cultivation of the agent. The virus is well cultivated without a reduction of pathogenic properties on fertilized chick embryos infected through the chorioallantoic membrane and the yolk sac, and also on media with fresh tissue, and in tissue cultures.

Resistance of the agent. The virus is relatively

resistant to heating and to the effect of disinfecting solutions. With a temperature of 60 degrees the viral suspension is completely inactivated in 10 minutes; a 0.1% formalin solution and a 0.5% phenol solution exhibit a destructive action after 24 - 36 hours.

In the frozen state the virus can maintain its activity for two years, and with a temperature of four degrees concentrated suspensions do not lose the infective capacity for 10 - 20 days. The virus is preserved in an active state for several months with lyophilized drying with subsequent storage at low temperatures.

Means of specific prophylaxis and therapy. Methods of vaccination against psittacosis have definitely not been developed. In the United States of America attempts are under way to produce a dead psittacosis vaccine.

Specific therapy is accomplished with antibiotics; aureomycin, chloramphenicol - and in the absence of a response - penicillin. The length of virus treatment with antibiotics is 10 days.

Clinical and laboratory diagnosis. The clinical diagnosis of psittacosis, especially in the first days of illness, is very complex. In the differential diagnosis it is necessary to exclude lobar pneumonia, influenza pneumonia, grippe, Q fever, the pulmonary form of plague, typhus, and typhoid fever.

Final confirmation of the clinical diagnosis is secured by laboratory findings. It includes the isolation and identification of the virus, determination of the complement fixation reaction with the patient's blood serum, and intradermal tests.

The isolation of the virus is done by means of infecting white mice. The material for inoculation is the patient's blood taken during the first 7 - 8 days of illness, and also sputum, pleural fluid, and pharyngeal washings in which the virus can be detected from the first day until the end of the illness.

The microscopic examination of the sputum has a definite value in the diagnosis. Viral elementary bodies can be demonstrated in smears stained with Romanovsky-Giemsa stain.

Complement fixing antibodies appear in the serum of patients starting from the 4 - 8th day of illness. In vigorous therapy with antibiotics the appearance of antibodies is maintained for 20 - 40 days.

The skin test becomes positive on the 3 - 4th day of illness, but it gives sharper results at the end of the second week.

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In the papers of Kliewe (4), Phair (5), and other foreign authors the psittacosis virus is mentioned time and again as a potential agent of bacteriological warfare.

In no instance in the foreign literature devoted to bacteriological warfare has there been a rejection of the possible use of the psittacosis virus for military purposes.

Rosebury and Kabat (2) believe that "the psittacosis virus is one of the most practical agents for the conduction of bacteriological warfare since it has great infecting capacity for man, is easily transmitted through the air, and can be obtained in large quantities."

### Smallpox

Smallpox is a serious, highly contagious illness for which there is a characteristic nodular-vesicular eruption on the skin and mucous membranes and a typical fever.

Pathogenicity of the agent. Man is very susceptible to smallpox. Epidemiological practice shows that infection can occur after a very short contact.

Infection occurs through the respiratory tract by the air-droplet and air-dust methods and also by means of contact.

Symptoms of the disease. The incubation period averages 14 days. At the end of the incubation period the illness starts up suddenly. It differs in severity by; average severity - variola vera, severe or black smallpox - variola nigra, and mild - variola minor.

The duration of the illness in uncomplicated cases is 5 - 6 weeks. Mortality reaches 25 - 30%, and death ensues at the end of the second week.

Smallpox is extremely contagious and is marked by the ability for epidemic spread; the patient is infective from the first day of the prodromal period right up to the falling off of the scabs (40 days). Patients require compulsory hospitalization. In isolation units it is necessary to observe a rigid antiepidemic regime to prevent the infection of personnel.

Methods of cultivation of the agent. The smallpox virus can be cultivated on fertilized chick embryos and

in tissue culture.

Resistance of the agent. The resistance of the smallpox virus, in contrast to the vaccinia virus which is similar to it, has been insufficiently studied yet. Epidemiological experience indicates that the virus reveals a high degree of resistance to various factors of the external environment.

The smallpox virus tolerates drying well by various methods and particularly by the method of lyophilization.

Means of prophylaxis and therapy. A live smallpox vaccine, which creates a high degree of non-susceptibility in man, is used for specific prophylaxis. The vaccine against smallpox is generally considered one of the most effective developed at the present time. Vaccination with a single inoculation is followed by revaccination.

Vaccination performed at the onset of the illness cannot deter it or moderate its course.

The matter of specific therapy of smallpox is different. As yet there are no specific therapeutic agents, and all therapeutic assistance to the smallpox patient is derived from symptomatic treatment.

Clinical and laboratory diagnosis. The diagnosis of smallpox during an epidemic is relatively simple. However, in the recognition of the first case of illness the clinical diagnosis of smallpox often presents great difficulty, especially at the onset of the disease before the appearance of the rash, since the initial stage resembles the prodromal stage of many infectious diseases.

Very often there are errors in the differential diagnosis of smallpox in children from chickenpox and measles.

Of the laboratory diagnostic methods two receive the most emphasis: the Paul test in rabbit cornea and Paschen's viral inclusion bodies. In recent years a number of new methods have been introduced: isolation of the virus by means of cultivation on the chorioallantoic membrane of chick embryos and the complement fixation reaction, which can be used for early (detection of antigen) as well as for retrospective diagnosis (detection of antibody). The method is rendered by detection of the virus with fluorescent sera.

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The question is repeatedly posed in the foreign literature about the possible use of the smallpox virus as an agent of bacteriological warfare (5, 6, etc). In



favor of this there are cited the basic characteristic features of smallpox: severity of the disease, high fatality, exceptional contagiousness and, consequently, the tendency toward a rapid epidemic spread.

At the same time some foreign authors have expressed grave doubts about the potential use of the smallpox virus for purposes of bacteriological warfare, and sometimes they have even rejected such a possibility altogether (27, 4). The basic arguments of this viewpoint are the presence of a highly effective vaccine and the wide conduction of specific prophylaxis of smallpox.

### Epidemic Equine Encephalomyelitis

Many forms of epidemic equine encephalomyelitis are now known. However, only a few of them represent a danger to man. Among these encephalomyelitis strains belong: American eastern equine encephalomyelitis, American western equine encephalomyelitis, and Venezuelan equine encephalomyelitis.

As possible types of a bacteriological weapon most attention abroad is turned to agents of American equine encephalomyelitis (2, 4, etc).

Pathogenicity of the agent. The viruses of American equine encephalomyelitis display a comparatively high pathogenicity for humans; however, the agent of eastern encephalomyelitis is still more pathogenic to man.

Along with the passage of the virus by the transmissible route through bites of infected mosquitos and ticks, cases have been reported in the literature of intralaboratory infections (42, 43, etc). Infection in laboratories occurs by the air-droplet route in work with the material containing the virus.

Symptoms of the disease. The incubation period of western encephalomyelitis is 5 - 10 days; it has not been established for eastern.

The onset is sudden, the temperature quickly rises to 40 - 41 degrees; a disturbance of the central nervous system, severe headache, insomnia, and vomiting are experienced. Eastern encephalomyelitis is especially severe with the development of convulsions, rigidity of the neck muscles, and paralysis of the extremities. The acute symptoms of the illness last from several days to three weeks.

The mortality rate in western encephalomyelitis is 8 - 20%, in eastern it reaches 74%.

Very often various residual manifestations are sustained in convalescents ranging from emotional instability to different types of paralysis and mental disturbance.

The disease is noncontagious.

Methods of cultivation of the agent. The viruses grow well on developing chick embryos and in tissue culture. A method has been described of the cultivation of the virus of eastern encephalomyelitis on the embryos of mosquito-fish (44).

Resistance of the agent. The viruses of American equine encephalomyelitis have pronounced resistance. Thus, for example, the virus of western encephalomyelitis can be maintained for a long time in a 0.5% phenol solution and in a 0.05% corrosive sublimate solution, and it is preserved well in the cold with a 50% glycerin buffer with a pH = 7.4 - 7.5 and in the frozen state as well. In media with a pH below 6.5 the virus is rapidly inactivated.

The viruses tolerate high temperatures poorly and are destroyed at a temperature of 60 degrees in 10 minutes.

Means of specific prophylaxis and therapy. For specific prophylaxis in horses there is now used in the USA a formalized vaccine prepared from a virus cultivated on chick embryos. The vaccine has also been tested in people. After a two-stage intravenous vaccination specific antibodies have been detected in the immunized individuals. The general reaction from the injection of the vaccine has been moderate.

A prophylactic bivalent formalized vaccine for western and eastern encephalomyelitis is now being made in the USA (45). However, an appraisal of the effectiveness of the vaccine for human immunization requires more testing under epidemiological conditions, which is pointed out in the foreign literature.

Foreign authors have this to say about the treatment of the disease. The administration of hyperimmune serum has not given satisfactory results. Sulfanilamide preparations are ineffective. There is no information about therapy with antibiotics in the foreign literature.

Clinical and laboratory diagnosis. To establish the clinical diagnosis of American equine encephalomyelitis, excluding encephalitis of other etiology, particularly St. Louis encephalitis, Japanese, tick, and others, presents quite a problem. In connection with this, the diagnosis of the two forms of American encephalomyelitis can positively only be established by means of laboratory

methods directed at the isolation of the virus and serological analysis.

The most suitable method of isolation of the virus from man is by the intracerebral injection of white mice. The best period for the isolation of the virus from the patient's blood is from the 3rd to the 7th day of the illness. At a later time and at the onset of the illness the virus cannot be isolated from the patient's blood as a rule.

Detection of specific antibodies is accomplished by two methods: the complement fixation and neutralization reactions. Complement fixing antibodies appear at the end of the first week of illness and viral neutralizing ones after a month.

From the presented data it is seen that the laboratory and clinical diagnosis of American equine encephalomyelitis presents great difficulty.

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Encephalitis and encephalomyelitis of viral etiology attract a great deal of attention abroad as potential means of bacteriological warfare. Some authors (4, 6) do not elaborate what types of encephalitis they have in mind, and others express themselves more explicitly, giving preference in this situation to American equine encephalomyelitis (2).

## Yellow Fever

Yellow fever is an acute (endemic) infectious disease transmitted under natural conditions by mosquitos of the genus *Aedes aegypti* and characterized by high mortality.

Pathogenicity of the agent. The virus has high pathogenicity for man. Under natural conditions the only carriers of the virus are mosquitos. However, the virus can also be transmitted without the insect carriers, and numerous publications of foreign authors refer to this. Berry and Kitchen (25) have described seven incidents of laboratory infection connected with the yellow fever virus and they have made a resume of 34 laboratory infections with five fatal outcomes. Some of these cases were caused by infected mosquitos, but a larger part arose without

any connection with mosquitos. A few cases of infection have been observed in persons engaged with the active drying of the virus. A very interesting case was one where the subject was in the laboratory for only one day and merely helped in the work with the infected material for a few minutes.

On the basis of an analysis of the factual material on intra-laboratory infections Rosebury and Kabat (2) have expressed a very definite opinion about the possible transmission of the yellow fever virus by the airborne route.

Symptoms of the disease. The incubation period usually consists of 3 - 6 days. The onset is abrupt. The febrile curve has a typical "saddleback" form: the first wave with a temperature of 39 - 40 degrees lasts for 3 - 4 days and then a remission follows lasting from several hours to 2 days and is replaced by a high temperature again lasting 3 - 4 days.

The condition of the patient is extremely serious. A severe headache and pains in the muscles and bones develop. The illness is marked by the development of jaundice and hemorrhagic diathesis with profound intoxication, and this is manifested by a yellow discoloration of the skin, mucosas, and sclera, bleeding from the nose and gums, "black vomit", and tarry bloody stools. The urine reveals pronounced albuminuria, the pulse indicates extreme bradycardia.

In addition to the severe forms of yellow fever there are also encountered mild, abortive forms.

The mortality in yellow fever averages 5 - 10%, rising in a severe epidemic to 20 - 25%. Death most often comes between the 5th and 9th day of illness.

Complications are rarely observed in yellow fever.

The period of convalescence is of short duration as a rule and ends with complete recovery.

The sick person does not present a danger to the community. However, during the first 6 - 7 days of fever the patient should be in a room which is inaccessible to mosquitos.

Methods of cultivation of the agent. The virus grows well on fertilized chick embryos with infection in the amniotic sac or chorioallantoic membrane, and in tissue cultures. However, strains of the virus, which do not adapt to chick embryos, are capable of producing infection in them only with the introduction of large doses of the agent. While the virus is being subjected to extended passages both in chick embryos as well as in tis-

sue culture, it partially loses its virulence and its pantotropic affinity.

Resistance of the agent. The virus is very labile: it is easily inactivated at high temperatures and with the common antiseptics. The heating of liquid cultures to 60 degrees renders them harmless in 10 minutes. Disinfecting solutions rapidly kill the virus.

In the dried state the virus has considerably greater thermal resistance, and it withstands low temperatures well also. The material containing the virus preserves its activity in 50% glycerin for 100 days at a temperature of 0 degrees.

Means of specific prophylaxis and therapy. Active, specific prophylaxis in yellow fever is accomplished by means of immunization with live vaccines. At the present time two attenuated strains - French neurotropic ("Dakar") and the American strain 17D - are used in the preparation of the vaccines.

Available data indicate the high effectiveness of the administration of a vaccine for yellow fever. The mass vaccinations, which have been conducted abroad, have given completely satisfactory results.

Specific therapy for yellow fever has not been developed. The application of all known therapeutic preparations has not been successful.

Clinical and laboratory diagnosis. The early clinical diagnosis of yellow presents some difficulties. It is necessary to perform a differential diagnosis from acute yellow atrophy of the liver, ictero-hemorrhagic leptospirosis, epidemic hepatitis, malaira, relapsing typhus, hemorrhagic fevers, and especially dengue fever.

For laboratory confirmation of the diagnosis of yellow fever three methods are used primarily: 1) isolation of the virus; 2) detection of viral neutralizing antibodies in the patient's blood; 3) patho-histological examination of the liver in case of a fatal outcome.

For isolation of the virus white mice are injected intracerebrally with the patient's blood taken up to the 5th day of illness. The neutralization reaction gives positive results beginning with the second week of the illness and can be used only for retrospective diagnosis.

The complement fixation reaction, due to negligible sensitivity, is not widely used.

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Many foreign specialists consider the yellow fever

virus the most promising type of bacteriological weapon, answering many of the requirements made of agents of bacteriological warfare (2, 19). Dissemination of the virus by the airborne route has attracted special attention. The possible transmission of the yellow fever virus through infected mosquito carriers has not been ignored.

### Dengue Fever

Dengue fever is an acute viral disease transmitted under natural conditions by mosquitos and characterized by febrile episodes, severe pains in the joints and muscles, an eruption, and leukopenia.

Pathogenicity of the agent. The infectivity of the virus apparently is very high, since during epidemic outbreaks in several areas from 75 to 100% of the inhabitants were afflicted.

Under natural conditions dengue fever is transmitted only through bites of mosquitos. An attempt to transmit the virus to man by means of rubbing the infectious material into scarified skin and the mucous membrane of the nose gave negative results (2).

Symptoms of the disease. The incubation period usually lasts for 5 - 8 days, rarely up to 15 days. In approximately half the cases a prodromal period precedes the illness, and in the other half it begins suddenly by a rapid elevation in temperature and chills. The temperature reaches 39 - 40 degrees on the very first day and stays there for 4 - 5 days. After 1 - 2 days of remission the temperature again rises. The second episode lasts for 2 - 3 days. Prognosis of the illness is favorable, complications are very rarely encountered. Fatality even in the period of large epidemics does not exceed 0.5%. It should be mentioned that very frequently a lingering convalescence is encountered in which there is observed a marked weakness.

Thus, dengue fever belongs to the group of comparatively mild illnesses. The sickness is not transferred from a patient to a healthy person.

Methods of cultivation of the agent. The virus of dengue fever grows poorly. On chorioallantoic membrane of fertilized chick embryos there are cultivated only strains which are adapted to mice. Therefore, recovery of the virus in large quantities obviously presents a serious problem.

Resistance of the agent. The virus possesses considerable resistance. Ultraviolet radiation for 30 minutes and the action of a 0.1% formalin solution for five hours does not inactivate it completely. With a temperature of -70 degrees or in the dried state the virus can be preserved for five years, with a temperature of 4 degrees - for several weeks, in blood serum of the patient at room temperature - two months.

Means of specific prophylaxis and therapy. An effective vaccine against dengue fever has not yet been developed. In recent years abroad some attempts have been made to prepare a live vaccine from mouse brain infected with an adapted strain of virus, but it seemed that it gave short-term immunity and therefore until now has not been widely used.

Specific therapy for dengue fever has not been developed.

Clinical and laboratory diagnosis. The diagnosis of dengue fever at the peak of an epidemic is not very involved and is established by a sudden onset of the illness, marked muscular and joint pains, a two-phase temperature curve. It is considerably more difficult to correctly determine dengue fever in sporadic, atypical, and primary cases. In the establishment of a diagnosis here it is necessary to have in mind malaria, relapsing typhus, measles, pappataci fever, grippe, and yellow fever in particular.

The laboratory diagnosis of dengue fever is insufficiently developed and has not found wide practical application yet. Isolation of the virus from the patient's blood by means of the intracerebral injection of suckling mice is possible only in the first 2 - 3 days of the febrile period.

The complement fixation reaction, neutralization reaction with sera taken from patients in the febrile and convalescent periods, and skin tests have been developed in recent years.

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Many foreign experts consider that the dengue fever virus satisfies many of the requirements of bacteriological warfare (2, 5, etc). In addition, it has repeatedly been observed abroad that the complexity of cultivation of this virus considerably hampers its possible application.

Summarizing the question about the use of the dengue fever virus for purposes of bacteriological warfare, Rosebury and Kabat (2) wrote: "Apart from the unsolved question about the possibility of obtaining a sufficient amount of the dengue virus for its use in bacteriological warfare, the properties of this agent characterize it as one of the suitable bacteriological agents. The practical value of this agent must be determined by further experimental studies, particularly experiments determining the possibility of preserving the virus in a virulent state and a study of the means of protection of the armies using this virus as a means of attack. The existing data compel us to assume that both of these problems can be favorably resolved."

## RICKETTSIAL INFECTIONS

### Typhus

Epidemic typhus is an acute infectious disease characterized chiefly by involvement of the vascular system and disposed to a rapid epidemic spread in the presence of the louse vector.

Pathogenicity of the agent. Infection with typhus under natural conditions occurs as a result of rubbing the excrement from infected lice into the abraded skin at the site of their bites. However, the illness can apparently arise as a result of the contact of infectious material with the conjunctivae and the respiratory tract.

Minimal infecting doses of the agent are not known for man, but epidemiological practice has reason to believe that they are trifling.

Symptoms of the disease. The incubation period lasts for 10 - 14 days with fluctuations from 6 - 23 days. The illness begins suddenly. The temperature quickly rises (38.4 - 40.5 degrees) and remains at this level until the end of the illness; the temperature subsides by rapid lysis. The general condition of the patient is often serious, and during the entire period of illness the patient requires careful nursing care. At the onset of the illness there is noted hyperemia and puffiness of the face, injection of the vessels of the sclera and conjunctivae.

The illness lasts for 10 - 14 days and is followed by a period of convalescence lasting about 10 days during



which the patient is incapable of working. Consequently, even in the absence of complications typhus incapacitates the patient for a long time.

With a timely start of specific antibiotic therapy the length of the febrile period is shortened, but the date of discharge (12 days after the temperature returns to normal) remains as before.

Before the discovery of antibiotics typhus mortality, depending on the age of the patient, ranged from 10 to 50%.

The application of modern methods of treatment with antibiotics almost excludes fatal outcomes.

In the absence of vectors the disease is not transmitted from person to person, but in the presence of lice typhus is prone to epidemic spread.

Methods of cultivation of the agent. Rickettsiae are well cultivated on fertilized chick embryos without loss of pathogenic properties. Therefore, the recovery of cultures in large amounts is not difficult.

Resistance of the agent. The rickettsiae of typhus exhibit comparatively little resistance to various factors of the external environment. In heating a suspension at 56 degrees the rickettsiae are rendered harmless in 30 minutes. In liquid suspensions at room temperature the agent dies in a day.

With low temperatures and humidity the rickettsiae in some substrates (excreta of lice) can be preserved for several days. Typhus rickettsiae are well preserved with dessication from the frozen state and can be stored for a long time under vacuum. The typhus agent dies quickly with the action of disinfecting substances.

Means of specific prophylaxis and therapy. Several vaccines are available for typhus prophylaxis. The most effective is one prepared from the yolk sac of infected chick embryos. Vaccination does not prevent the illness but it ameliorates its course appreciably. Persons who have been vaccinated usually recover.

In recent years a new live vaccine has been developed abroad from the avirulent strain E, and it has proved quite effective.

Antibiotics are used as a means of specific therapy aureomycin (chlormycin), levomycetin (chloramphenicol), and syntomycin.

Laboratory and clinical diagnosis. The clinical diagnosis of typhus in the first three days of illness is very difficult. A typical rash appears on the 4th - 6th day of illness, and this facilitates a diagnosis.

The laboratory tests do not aid in an early diagnosis.

Diagnosis by means of serological reactions can be established only from the end of the 2nd week of illness. Methods of isolation of the agent are laborious and require a long time -- 20 - 25 days.

The opinions of foreign specialists about the possible use of the typhus agent as a means of the bacteriological weapon are very controversial. Rosebury and Kabat (1947) (14) put typhus in the group of infections whose agents were omitted from the study for military purposes. The authors tried to base their opinion on the fact that typhus can have broad epidemic spread only in the presence of the necessary predisposition of social factors. A similar opinion was held by Keane (1948) which in general considered that the discovery and wide usage of DDT and other highly effective synthetic insecticides helped rather easily to combat all infections transmitted by insects. Therefore, he felt that in the presence of modern means of control typhus will not have a broad epidemic spread and scarcely can be used for military purposes.

However, in recent years this viewpoint has not found support in the foreign literature. On the contrary, many foreign specialists (Phair, 1954 (3); Bullene, 1954 (16a); Creasy, 1955 (6); Stevenson and Berger, 1955 (3)) have very explicitly stated the possibility of using the typhus agent as a means of bacteriological warfare.

### Rocky Mountain Spotted Fever

Rocky Mountain spotted fever is an acute infectious disease characterized by a natural focus and transmitted under natural conditions by the black-legged ticks.

Pathogenicity of the agent. Rickettsiae of spotted fever are highly pathogenic for man. A primary route of infection in man under natural conditions is the bite from an infected tick vector, but that may not exclude the possibility of the disease arising also with contact of the contaminated material (for example, fluids from crushed ticks) with the mucous membrane and the skin. It has been shown in experiments on animals that infection is successful with contact of the rickettsiae on the conjunctivae and even on the intact skin (46).

Symptoms of the disease. The incubation period lasts on an average of 6 - 7 days. In some cases it can

vary from 2 - 3 to 14 days. There is no initial reaction at the site of the recent tick bite in the absence of other tick rickettsiae. Usually the illness begins suddenly. The temperature quickly rises to 40 - 41 degrees and remains elevated for an average of 2 - 3 weeks. The patient develops very severe headache, vomiting, insomnia, disturbance of consciousness. Convulsions, pathological reflexes, paralysis of the cranial nerves, para- and hemiplegia are manifested. From the 3 - 4th day of illness the spleen can be palpated.

On the 2 - 4th day of illness (sometimes on the 5 - 6th) an eruption appears, manifested at first as a rose rash, then a maculo-papular, and in serious cases - hemorrhagic. At first it appears on the ankles and wrists, but it soon covers the skin of the hands, legs, chest, and sometimes the face and hair. The skin of the abdomen is affected least of all. The illness is often complicated by thrombophlebitis, necrosis of the integument, weakening of eyesight and hearing, and paralysis.

Mortality ranges from 5 - 10 to 80%. The introduction into practice of antibiotics has significantly reduced these figures.

Transmission of the agent from person to person has not been observed, and therefore the patient does not represent a danger to the community.

Methods of cultivation of the agent. The agent, like other members of the rickettsial group, does not grow on synthetic nutrient media without the presence of living tissue, but it is well cultivated on fresh and transplanted tissues, especially on the yolk sac of developing chick embryos.

Resistance of the agent. The resistance of rickettsiae of Rocky Mountain spotted fever to the effect of unfavorable conditions of the external environment is not high. They die within a few minutes in a moist environment at a temperature of 50 degrees or with the action of disinfecting substances. Dried rickettsiae die in several hours at room temperature.

Means of specific prophylaxis and therapy. Two forms of killed vaccine are offered for vaccination against Rocky Mountain spotted fever: one is prepared from the tissue of infected ticks, the other from rickettsiae grown on the yolk sac of fertilized chick embryos.

Vaccination is performed subcutaneously in three steps with later revaccination.

Vaccination usually insures complete protection from slightly virulent strains but is rather less effec-

tive in respect to strains with high virulence. However, even in these cases it significantly moderates the severity of the course and reduces the number of fatal cases to zero.

In the USA an avirulent rickettsial strain was obtained as a result of prolonged passages on chick embryos. It is possible that in the future it can be used in the preparation of a vaccine.

Specific therapy of the illness is accomplished by antibiotics: aureomycin and terramycin. The course of treatment with antibiotics is 5 - 6 days. However, clinical improvement is observed after twenty-four hours and a drop in temperature after 60 - 70 hours.

Clinical and laboratory diagnosis. The clinical diagnosis of Rocky Mountain spotted fever, notwithstanding the prevailing opinion, often is not simple. The diagnosis is especially complicated in those areas where rat typhus and other rickettsial diseases, which have many features in common with the given illness, are encountered along with spotted fever.

As in typhus, the laboratory diagnosis does not assist in early recognition of the illness.

Isolation of the agent by intraperitoneal injection of male guinea pigs consumes much time.

The Weill-Felix reaction has non-specific importance since it only indicates that the illness belongs to the rickettsial group. Specific tests are the complement fixation and the neutralization reactions which aid in the identification of the illness and have great value for retrospective diagnosis.

In late years the use of the complement fixation reaction with the application of a standard specific antigen of rickettsiae has been satisfactorily instituted for the serological diagnosis of spotted fever.

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Recently isolated reports have appeared in the foreign literature concerning the potential use of rickettsiae of Rocky Mountain spotted fever as a bacteriological weapon (2, 5, 6).

Rosebury and Kabat (2) believe that the basic method of dissemination of the spotted fever agent in bacteriological warfare will be the use of infected ticks of *Dermacentor andersoni* species. The possible application of

other species of tick vectors - Rhipicephalus and Haem-physalis - has not been excluded. These same authors have expressed the opinion that an area, which is subjected to attack for a long time, will be dangerous in the sense of infection resulting from the presence of infected ticks.

### Tsutsugamushi Fever

Tsutsugamushi fever, or Japanese river fever, is an acute infectious disease, spread under natural conditions through the bites of larvae of some types of the trombiculid mite, and characterized by a natural focus.

Pathogenicity of the agent. Rickettsiae of tsutsugamushi fever possess a high pathogenicity for man. The agent is transmitted to man normally only through the bites of larvae of some species of the trombiculid mites. Cases have been described in the literature of intra-laboratory infections of tsutsugamushi fever due to the contact of infectious material on the conjunctiva (47)

Symptoms of the disease. The incubation period is generally 10 - 12 days. The disease begins suddenly, the temperature reaches maximal figures (40 - 40.5 degrees) by the end of the first week of illness and remains at this level for 2 - 3 weeks. The classical symptoms are the primary reaction at the site of the bite of the vector and generalized lymphadenitis. Between the 5th and 8th day of illness a rash appears which at first has a macular and then a maculo-papular character. It appears on the chest and abdomen and then spreads all over the trunk and extremities, and sometimes to the face also. Foci of pneumonitis develop in many patients. The illness is accompanied by severe headache, and in severe cases manifestations of meningoencephalitis develop. At the onset of the illness the peripheral blood reveals a noted leukopenia and lymphocytosis.

Convalescence starts at the end of the second or beginning of the third week. The period of convalescence is usually rather prolonged.

Prognosis in the illness varies markedly. Mortality can fluctuate in very broad ranges - from 1 to 60%. These deviations are related to the singularities of the epidemic focus as well as to the age of the patient. A high mortality rate is noted among elderly patients. Death usually sets in at the end of the second week of illness.

All of these facts pertain to that period when there were no specific preparations for treatment. With the introduction into practice of antibiotics the prognosis has significantly changed, and fatality amounts to practically zero.

A patient with tsutsugamushi fever is not a danger to the community, and the illness is noncontagious.

Methods of cultivation of the agent. Rickettsiae are well cultivated on fertilized chick embryos with infection in the chorioallantoic membrane or the yolk sac. In addition, the rickettsiae of tsutsugamushi fever can also grow on Zinzer's tissue-agar medium (48).

Resistance of the agent. Rickettsiae are characterized by great lability and low resistance to the external environment. Heating to 50 degrees inactivates them in 10 minutes. Rapid destruction occurs in a 0.1% formalin solution and a 0.5% phenol solution. The agent can be preserved for a long time at a temperature of -70 degrees. Lyophilized drying, even with careful adherence to all the requirements, results in an appreciable lowering of the infective titer (49).

Means of specific prophylaxis and therapy. Attempts to devise effective vaccines have not yet been successful. In the USA two types of dead vaccines have been prepared - from tissue-agar cultures and from the lungs and spleens of infected white rats. Both vaccines have proved ineffective in epidemiological experiments (50). Then a method of vaccination has been tried with a live strain accompanied by the administration of chloromycetin (48). It is very obvious that because of the complexity and inefficacy this method can be applied only under special circumstances.

To prevent the emergence of tsutsugamushi fever Smadel and other authors (48) have suggested using chemoprophylaxis by the administration of chloromycetin. Antibiotics have been used with great success in the treatment of the disease: aureomycin, terramycin, chloramphenicol.

Patients, who respond to the antibiotics, recover quickly and by the 10th - 14th day following the end of the febrile period can return to work if they do not attempt undue physical exertion.

Clinical and laboratory diagnosis. The clinical diagnosis of tsutsugamushi fever often presents quite a problem, because such symptoms as headache, relative bradycardia, and absence of leukocytosis can be encountered with a number of other illnesses which are found in the

very same localities - dengue fever, malaria, typhoid fever, and other rickettsial diseases.

The most accurate method of laboratory diagnosis is the isolation of the agent from the patient's blood during the febrile period by means of intraperitoneal infection of white mice. In the presence of tsutsugamushi fever rickettsiae in the test material the mice die on the 10 - 18th day after injection.

The serological diagnosis consists of the Weil-Felix reaction using the *B. proteus* X<sub>k</sub> antigen. Antibody in the patient's blood serum appears by the end of the second week of illness.

The complement fixation reaction with rickettsial antigen is not used because of the serological variations in the agent of the given species.

Thus, as in other rickettsial diseases, the clinical and laboratory diagnosis of tsutsugamushi fever presents a certain difficulty which to quite an extent limits the possibility of early recognition of the illness.

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The agent of tsutsugamushi fever was not mentioned in the foreign literature of the 30's and 40's as a possible agent of bacteriological warfare, and it also was not considered in the well-known investigations of Rosebury and Kabat (2). However, in recent papers abroad devoted to questions of bacteriological warfare the rickettsiae of tsutsugamushi fever have repeatedly been discussed as a potential type of bacteriological weapon (6).

#### Q Fever

Q fever is an acute febrile illness marked by the involvement of the vascular and reticulo-endothelial systems.

Pathogenicity of the agent. Infectivity of Q fever rickettsiae is very high for man, and this is indicated by the numerous cases of intralaboratory infections. Dwyer and associates (51) observed an outbreak of Q fever in one of the buildings of the National Institute in the USA where the possible transmission of the agent through the air was established.

Infection in man can occur by the alimentary route in

addition to the airborne route.

Symptoms of the disease. The incubation averages 15 - 20 days. There is no primary reaction. The onset of the illness is acute. The temperature quickly rises (38 - 40 degrees). The febrile period usually continues for 1 - 2 weeks, sometimes three weeks. The principal complaints are severe headaches and muscular pains. The characteristic symptom of the illness, especially with the airborne route of infection, is the specific involvement of the lungs in the form of rickettsial pneumonia. With a timely start of antibiotic therapy the temperature drops to normal in 3 - 4 days. A larger number of patients require hospitalization for 10 - 15 days.

Complications of any kind, as a rule, are not observed in Q fever. Mortality in this illness is negligible and is noted only among patients of the older age groups.

Q fever belongs to the group of noncontagious illnesses, and sick people do not present a danger to the surroundings.

Methods of cultivation of the agent. Q fever rickettsiae are easily cultivated in chick embryos with infection in the yolk sac. The agent can also be successfully propagated in vitro on media with transplanted tissue.

Resistance of the agent. Rickettsiae of Q fever are preserved very well and for a long time in the external environment, and also in moist as well as in dry substrates.

Thus, the blood of infected guinea pigs, overlaid with a mixture of vaseline and oil, preserves its infectivity for animals for more than nine months (52). With storage in the refrigerator the rickettsiae remain viable for 41 days in butter made from the milk of infected cows (53).

The agent of Q fever survives drying. Thus, the dry defecation of infected mites with its maintainance at room temperature exhibits infectivity for guinea pigs for 586 days (54). In addition, the dried urine and blood of infected animals can maintain virulence for several weeks.

Means of specific prophylaxis and therapy. A dead vaccine, prepared from egg cultures of rickettsiae, has been developed for the specific prophylaxis of Q fever. It is recommended that a three-stage vaccination be used with additional booster shot after 4 - 5 months (55, 56).

Aureomycin and levomycetin are effective agents for specific therapy.

Clinical and laboratory diagnosis. The extremely



diversified clinical forms of Q fever make it very difficult to establish an early clinical diagnosis. Therefore, the laboratory diagnosis assumes special importance and includes basically two methods: the isolation of the agent and the establishment of the serological reactions.

The isolation of the rickettsiae is accomplished by means of intraperitoneal infection of guinea pigs with the patient's blood taken during the febrile period.

The serological diagnosis is performed with specific antigen prepared from egg cultures of the rickettsiae.

Agglutins appear in the patient's blood by the end of the first week of illness. Complement fixing antibody is detected from the 7th to 30th day following onset of the illness.

From the cited data it is seen that the laboratory methods do not help to establish the nature of the illness in the early period.

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Rosebury and Kabat (2) consider that "rickettsiae of Q fever apparently can be applied for bacteriological warfare but with some limitations."

Similar views have also been expressed in recent years by Phair (5), Berger and Stevenson (6) and other foreign authors.

## FUNGUS DISEASES

### Coccidioides

Coccidioides is a deep mycosis of man; it is caused by the yeast fungus, *Coccidioides immitis* and is characterized by a great variety of clinical forms.

Pathogenicity of the agent. The fungus has high infectivity for man, which is proved by the numerous laboratory infections and also by the fact that in endemic areas almost 100% of the inhabitants are infected with coccidioides (57). The infection of new arrivals in endemic areas also occurs easily. During the Second World War among the personnel of the armed forces of the USA, located in endemic areas of coccidioides, several thousand clinically manifested cases were recorded (58).

Under natural conditions the reservoir of the fungus in nature, according to Emmons (59), is rodents. The fungus is projected into the external environment with the

excretions of patients in the form of chlamydospores and arthrospores which evidently ripen in the ground and are lifted into the air along with dust. An opinion exists that the intermediate host of the fungus, which gives rise to the transition into the mycelial stage, may be plants (60).

A person is usually infected by inhaling the ripened chlamydospores and arthrospores. Some authors also recognize the possibility of infection through the integument (60). Infection through the digestive tract is not excluded.

Symptoms of the disease. The incubation period averages 10 - 14 days. On the basis of the clinical course of coccidioides it is customary to divide it into three principal forms: asymptomatic primary infection, or sub-clinical form; manifested primary infection, resembling grippe or pneumonia; progressive disseminated coccidioides or coccidioidal granulomatosis.

The asymptomatic primary infection makes up 60% of all the cases of coccidioides.

The manifested primary infection begins suddenly; the temperature quickly reaches 40 degrees and remains at this level for several weeks. The prognosis of this form is favorable on the whole.

Progressive disseminated coccidioides comprises approximately 0.2% of all the cases. In this form the fungi from the primary focus are conveyed with the blood and lymph throughout the whole system, and wherever the fungus settles, secondary granulomatous growths develop. Mortality in this form averages 50%.

The illness is slightly contagious and is not transmitted directly from person to person.

Methods of cultivation of the agent. The fungus is easily cultivated on ordinary nutrient media.

Resistance of the agent. The fungus is rather resistant to unfavorable conditions of the external environment, and chlamydospores and arthrospores tolerate drying well and preserve viability for a long time in the dried state (61).

Means of specific prophylaxis and therapy. Vaccines have not been developed for coccidioides. Effective means of specific therapy have not yet been offered.

Clinical and laboratory diagnosis. The clinical diagnosis of coccidioides, because of the great variety of symptoms, is very involved. Many cases remain undiagnosed.

The most reliable identification of the illness is the isolation of the fungus, which is very easily done in the disseminated form from the pus, sputum, and pleural flu-

id of the patients. Of the other methods of diagnosis the skin test with coccidioidin, which is widely used at present, has the most practical value. Sensitization to the organism is detected from the 2 - 20th day after the onset of the illness and is present in almost all patients even with asymptomatic forms of the infection.

In most patients with coccidioidosis precipitins and complement fixing antibodies are formed. However, these antibodies appear with the greatest regularity only in severe forms of the disease.

### Nocardiosis

Nocardiosis is a deep mycosis of man which is caused by the fungus, *Nocardia asteroides*, and progresses with principal involvement of the lungs.

Pathogenicity of the agent. The fungus has exceptionally high pathogenicity for man. Human infection with nocardiosis occurs by a twofold route: either airborne or through the damaged integument. In the latter case the illness is characterized by the development of purulent processes enveloping the subcutaneous tissues, muscles, and bones.

Symptoms of the disease. The length of the incubation period has not been established since exposure to the infection can never be accurately ascertained.

Nocardiosis most often occurs in the pulmonary form. The symptoms of pulmonary nocardiosis often resemble those of pulmonary tuberculosis. The illness usually starts gradually with general malaise, weakness, emaciation.

The duration of the disease ranges from 2 - 3 months to 1 1/2 years. The prognosis in pulmonary nocardiosis is very unfavorable and always terminates in death in untreated cases. Incidents of intrahospital infection have not been described, and patients apparently are not a source of danger in the community.

Methods of cultivation of the agent. *N. asteroides*, like other representatives of the nocardia genus, are easily cultivated on simple nutrient media, liquid and solid. Therefore, the recovery of the agent in large amounts does not represent a serious problem.

Resistance of the agent. There are no explicit factual data on the resistance of the fungus to various influences of the external environment in the literature available to us. However, considering that in biological properties the nocardia stands very close to the actinomycetes and the agent of tuberculosis, its relatively high degree of resistance has to be assumed.

Means of specific prophylaxis and therapy. Vaccines have not yet been developed. Sulphanilamide preparations (sulfadiazine, sulfamerazine) in large dosages are successfully used as a means of specific therapy. The course of treatment is long -- 2 - 3 and sometimes even five months (62).

Clinical and laboratory diagnosis. The clinical diagnosis of nocardiosis is extremely difficult. In most of the cases described in the literature the diagnosis was established only in the terminal stage or even at autopsy.

The clinical signs of the illness are very similar to tuberculosis. The laboratory diagnosis of nocardiosis depends chiefly on the isolation and identification of the fungal agent.

In the examination of slides under the microscope it has to be kept in mind that in sputum *M. asteroides* can break up into bacillary elements which can simulate tubercle bacilli because of their acid-fastness.

\* \* \*

In the foreign literature of the 1940's on questions of bacteriological warfare the agents of fungal diseases were not considered as possible forms of the bacteriological weapon. Rosebury and Kabat (2) even expressed the opinion about their uselessness in this respect because of the apparent lack of pathogenicity for man.

However, in recent years this opinion has evidently been revised. Such specialists as Phair (5), and Berger and Stevenson (6) consider it quite possible to use agents of fungal diseases as a means of bacteriological warfare, and special emphasis is given to the deep mycoses, particularly nocardiosis and coccidioidosis.

\* \* \*

We do not presume to an exhaustive presentation of aspects of this chapter, but we have tried to generalize the fundamental views of foreign specialists, which are known to us from the published foreign literature, about the reasons for the selection of microorganisms for purposes of bacteriological warfare, and also about the requirements made of

them as potential agents of bacteriological warfare.

On the basis of the opinions of foreign authors we have made an attempt to compile a list of microorganisms which best satisfy, from their point of view, the requirements of the bacteriological weapon. In addition, some of the characteristics of these agents and the diseases caused by them have been presented.

In the rendition of these aspects we have not made an attempt to give a complete description of every disease, but we have tried somewhat more in detail to delineate those features of the microorganisms and their diseases which, in the opinion of the foreign specialists, will determine them as potential agents of bacteriological warfare.

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## CHAPTER V

### BASIC MEANS OF ANTI-BACTERIOLOGICAL DEFENSE

#### ORGANIZATION OF ANTIBACTERIOLOGICAL CIVIL DEFENSE IN THE UNITED STATES OF AMERICA

Along with the means and methods of actively engaging in bacteriological warfare, the problem of defense against the bacteriological weapon is being intensively worked out in the United States of America. It is being solved both through instituting new, more improved means of defense and along the line of developing its organizational and tactical foundations. During the past few years, along with numerous articles in journals and separate monographs devoted to problems of bacteriological warfare (1, 2, 3, 4, 5, etc.), several official instructional handbooks and manuals have appeared in the United States of America, in which the principles of defense against bacteriological warfare are explained and data on its organization in the United States are presented (6, 7).

According to American literature, providing that proper reorganization is enacted, the United States presently has sufficient means at its disposal to defend itself successfully against bacteriological attack. The United States system of anti-bacteriological defense (ABD) is based on the peacetime structure of Civil Defense agencies, as well as on the Departments of Public Health and Agriculture, which found their anti-bacteriological defense activities on the peacetime antiepidemic, sanitation and hygienic, antiepzootic and other measures directed against "natural" infectious diseases. However,



according to the unanimous opinion of the American specialists, under conditions of bacteriologic war these measures will be insufficient, and therefore they need substantial reinforcement and improvement.

The defense of population against bacteriologic warfare rests on the U.S.A. Civil Defense agencies, which direct the defense of the population of the country against all kinds of warfare. U.S.A. Civil Defense is directed by the Federal Administration, which includes Medical Service Administration, supervising all the problems of medical protection of the country under conditions of enemy attack with any kind of weapon. Medical Service Administration includes 4 sections: medical-evacuational, sanitary-hygienic, medical supply service and the service of defense against special kinds of weapons (atomic, chemical, bacteriologic). This last service includes, along with the divisions of Atomic and Chemical Warfare Defense, also the division of Antibacteriologic Defense.

One of the basic duties of the Federal Civil Defense Administration in the time of peace is the preparation of mobilization and operation plans "so that everyone would know his duties when an unusual situation arises" (6). Federal plan of organization of Medical Service in the sphere of defense against special weapons has already been worked out and it provides for 3 stages: 1) preparation of the country in peacetime; 2) measures to be taken during the use of special weapons; 3) elimination of the effects of the attack (6).

The federal administration is responsible for the formation and personnel training of civil defense, the publication of manuals, instructions and directions, the construction of shelters and auxiliary structures, the outfitting of laboratories and medical institutions, the provision of supplies required in case of war, etc. During and after the employment of special weapons, the federal government assumes general direction of defending population, domestic animals and crops; it is responsible for performing measures required after attack. Among the functions of the anti-bacteriological defense division are the planning and direction of all civil defense operations in the field of anti-bacteriological defense, as well as the coordination of parallel work carried out by the Departments of Public Health and Agriculture, and other departments. The special duties of the anti-bacteriological defense division are: the detection and identification of the bacteriological agent used, performing sanitation and antiepidemic work and decontamination (6, 7).

Seven civil defense regions were formed in the United States during 1953, which coincide with the area of the military districts. The civil defense administration of these regions direct the civil defense services of the constituent states.

There is no uniform structure of State Civil Defense in the U.S.A. It is adapted to the conditions and peculiarities of each State of the country. Generally, however, this structure resembles the organization of the Federal Civil Defense Administration. The States have special Health Departments, which include Divisions of defense against special weapons, with subdivisions of antibacteriologic defense. The State Health Commissioner has a Permanent Advisory Council of physicians, nurses, veterinaries, pharmacists, sanitation engineers and administrators. The functions of the Council include creation and corrections of the

mobilization program of the State Civil Defense Health Department, as well as assisting the Civil Defense Commissioner in the problems of rational use of available resources, coordination of local Civil Defense plans, keeping in liaison with Federal Civil Defense Administration and with the State Commissioner of Agriculture (6).

Organization of antibacteriologic defense within the Civil Defense system can be demonstrated on the example of the State of Maine, where it took 3 years (1950-1953) to build up its present structure, which is recommended by the Federal Administration as an example for other states (8).

General directions of Antibacteriologic Defense of the State of Maine rests on the Division of Special Warfare Defense, which is a part of the State Civil Defense Medical Service and includes the A.B.D. division.

The Director of the State A.B.D. Service has an advisory committee of physicians (microbiologists, parasitologists) and representatives of the Departments of Agriculture and River, Sea and Shore Fishing. The duty of the committee is to assist the Director in organizing the antibacteriologic defense of the State, and in particular, in supervising the organization and training of the local commands of bacteriologic detection.

The State is divided into 6 districts, each of them having its own District Diagnostic Laboratory, created at some scientific institution or a hospital. Each District Laboratory has two flying squads, the duty of which is the collection of samples and performance of the simplest analyses

in order to identify the bacteriologic agent and to direct the samples to the laboratory. The squad consists of one senior bacteriologist and three laboratory technicians (voluntary). In addition, the Health Service of the State organizes two mobile laboratories for the purpose of identification of bacteriologic and chemical weapons.

Along with the Mobile Laboratories performing the simplest duties of recognition of the bacteriologic weapon used, the State of Maine has a Central Diagnostic Laboratory (City of Augusta) to carry out the more complex bacteriologic investigations.

In addition to the duties of recognition of a bacteriologic weapon, all these Civil Defense Laboratories and Health organs are to take care of the wounded and the sick, and to conduct the sanitary-hygienic analyses.

Such scheme of the structural organization of the antibacteriologic defense of the State of Maine is approved by the U.S. Federal Civil Defense Administration.

In American cities and settlements, Local Civil Defense has been organized. It includes Medical Service, the specialists of which enter into Mobile Support Units. The Chief Medical Service Officer has also a committee of advisors, recruited from the local medical workers. In addition, Local Civil Service includes the Services of Reconnaissance and Information, evacuation, liaison, aid to the hit population, registration and information, as well as the Welfare, Fire and Police Services. Basic functional subdivisions of Local Civil Defense are specialized detachments and self-defense groups, recruited from urban and rural population.

During the time of peace, the self-defense groups study the rudiments of civil defense and train the voluntaries. During and after the attack on a city (settlement) they take part in the defense and evacuation of the population, supply first aid and dispose of the consequences of the attack (6, 9).

Cities, industrial areas and certain districts of major strategic importance are designated as target and special target areas. At present, there are in the U.S.A. 123 target and 70 special target areas, with a population of 67 million (10).

Basic tactical units of the Civil Defense are Mobile Support Units, created locally; one of their functions is carrying help to the attacked district. Such Units include both medical service and First Aid Stations, Surgical and Special Divisions and radiologic and bacteriologic survey services. Upon arrival into a stricken district, the medical service units report at the Medical Service Center and remain at its disposal (9).

An overwhelming majority of the ample manpower of the Civil Defense Medical Service is composed of citizens, who enter the Civil Defense ranks voluntarily. Certain number of posts, however, is filled in by the administration. Thus, particularly the Medical Service Directors are appointed from among the responsible workers of the Public Health organs (6, 9).

Where the problems of antibacteriologic defense are concerned, U.S. Civil Defense Service works in close contact with the Health Service and its organs, which carry the responsibility for the defense of population against bacteriologic warfare. Health Service Central Antiepidemic Department

is responsible for the detection and identification of bacteriologic weapons, organization of measures to prevent the infection of people and the spreading of disease and organization of treatment of the infected persons (11).

All the States have Departments of Health, which cooperate with the State Civil Defense. Local Health Service divisions function in collaboration with medical institutions and laboratories. They also remain in contact, collaborate with, and carry out orders of, the Local Civil Defense Service (4). In addition to this, in 16 states 181 U.S. Health Service District Divisions have been organized, which in the case of destruction of the central system can be used as provisional administrative centers of the State Civil Defense Medical Service.

State Health agencies and local Health divisions, at the time of peace, supervise the sanitary and antiepidemic measures, assure medical help and laboratory diagnosis service. In case of natural disaster, epidemic or bacteriologic attack their duties are as follows:

a) Fight against infectious diseases, including:

- epidemiologic detection to determine the source of the infection;
- organization of isolation and quarantine for persons in need of them;
- carrying out immunization of the population;
- carrying out disinfecting and disinsecting measures;
- cooperation with the local authorities in supplying medical

help and care to the sick;

- protecting the work of special laboratories.

b) Sanitary measures, providing:

- assurance of a safe water supply;
- assurance of food and medicine supply;
- control of sewage and removal of impurities;
- destruction of insects and rodents (5, 11).

The National Institute of Microbiology employs scientific methods in directing the antibacteriologic defense, as does the United States Institute of Health. To provide an epidemiologic detection service on a national scale, a special center for hygiene and infectious diseases has been established. The publication of data on the occurrence of infectious diseases is a duty of the National Bureau of Birth and Death Statistics (11).

The protection of productive animals and agricultural plants against bacteriological warfare is also carried out by the United States Civil Defense Administration, jointly with the Department of Agriculture. Problems related to the defense of animals are supervised by the Federal Bureau of Animal Husbandry, while those of plant defense are covered by the Bureau of Entomology and Plant Protection and the Bureau of Plant Production (6, 7).

In view of the fact that subversive methods of bacteriological attack are considered very plausible in the United States, the Federal Bureau of Investigation is included in the system of anti-bacteriological defense; its task consists in combating subversive agents (6, 7, 9).

It is assumed that troops of the United States armed forces will also participate in civil defense (12). Thus the Civil Defense Information and Observation Service is closely interrelated with the detection and interception system of the United States Air Force anti-aircraft defense. The support of the army will also be counted on in organizing anti-bacteriological defense procedures both during and after attack. It is considered that in order to strengthen the defense of

especially important regions and objectives, military groups in size of not more than a brigade will be created with the object of performing ground and air exploration, organization of welfare and restoration operations, fight against fires, provision of water and removal of obstructions.

In the U.S.A. particular attention is given to the problem of preparation of the ranks of specialists in the Civil Defense in general and defense against bacteriologic warfare in particular.

According to data presented at the annual report of Federal Civil Defense Administration for the year 1954, a Civil Defense Staff Command College was opened in April 1951 to train the chief workers of the administrative and operative agencies of civil defense. By December 1954, over 10,000 persons graduated from it. At this college, in addition to training personnel, the organizational foundations of civil defense are being elaborated together with teaching aids on this problem.

In 1951 and 1952, respectively, the Western (State of California) and the Eastern (State of Pennsylvania) schools of technical preparation were organized for the purpose of preparation of personnel to work in the States and on locations, with a uniform program of instruction. The persons who have taken a course of instruction in these centers are to prepare the personnel and instructors of the defense groups at the state level. These instructors, in turn, train local volunteers.

In addition to this, at the Staff Command College in 1954 there were organized travelling courses in Civil Defense, which carry their work at the universities in the different States. Civil Defense program is



studied in American high schools and colleges.

The personnel training program in civil defense includes general as well as special preparation. General preparation comprehends studies of basic problems of Civil Defense: principles of its organization and duties in peace and war, first aid, conducting of the stipulated "operations" to rescue the victims, etc. Special preparation is given in the fields of antiatomic, antichemical and antibacteriologic defense.

A system of special educational institutions is planned for the preparation of Civil Defense Medical Service personnel. Before their creation, Federal Civil Defense Administration had to organize, for training in the defense against bacteriologic warfare, three types of courses: for the administrative personnel of the State Health organs (general course of lectures), for physicians-specialists (special course of lectures) and for the personnel of the State Health laboratories (technical course of lectures). On top of this, the Bureau of Animal Husbandry organized a course of lectures for outlying veterinarians, and Federal Civil Administration - a course of lectures on the subject of defense of crops against bacteriologic warfare, for agricultural workers.

All the personnel of medical institutions in the U.S.A. is also included in the Civil Defense preparation system; instruction is carried out under the general supervision of the local Defense Director, and under a direct supervision of Hospital Directors.

It is proposed that all persons joining the ranks of Civil Defense Medical Service should undergo local courses of instruction in the

problems of antiatomic, antichemical and antibacteriologic defense, know how to administer first aid to the wounded and the sick, know the general principles of treatment of burns and wounds, as well as be acquainted with general problems of the organization and administration of Civil Defense, the study of which represents 50% of the total instruction time.

American Red Cross takes active part in instructing the members of Civil Defense Sanitary Service; it conducts the preparation of the nurses and instructs the general population in the principles of First Aid (10).

In 1954, Civil Defense in America had 4.5 million volunteers, among them 200,000 specially trained instructors and over 10,000 directing workers.

Preparation of the U.S.A. to bacteriologic warfare defense is carried out according to special programs of people defense and animal, plants and crop defense, which have been worked out by the Civil Defense Authorities together with the Departments of Health and Agriculture. These programs include the principles of antibacteriologic defense, as officially accepted in the U.S.A. (7).

Thus the acquaintance with the literature proves that during recent years in U.S.A. the problem of organization of the defense of population against bacteriologic warfare has been intensively worked upon; at the same time, the basic attention within this problem has been directed towards a rational organization of the State Civil Defense Service, of methodical, scientific direction, planning and unification of antibacteriologic defense measures, as well as the preparation of the personnel.

In conclusion, it should be noted that this review does not pretend to give a full and thorough treatment of the subject, since it was not possible to find more exhaustive information (especially concerning the last 2-3 years) on the state of A.B.D. in the U.S.A., in the literature available.

Fundaments of antibacteriologic defense organization contain a series of general directive principles. Above all, it is considered that at present the U.S.A. possesses effective means of prevention, limitation and elimination of infectious diseases which could be caused by bacteriologic warfare. It is further assumed that the whole A.B.D. system should be constructed on the basis of the principles of antiepidemic and sanitary-hygienic protection, existing at the time of peace, as well as on the basis of existing medical institutions. It is admitted, however, that these principles should be supplemented and the institutions extended and fortified. It is believed that the whole A.B.D. system should be constructed on the consideration that the enemy power will be able to use only the agents of presently known infectious diseases. At the same time, the necessity of preparation for the defense against all possible kinds and combinations of bacteriologic warfare is being stressed.

It is admitted that the enemy will use agents of diseases not found under natural conditions in a given location. It is supposed that the agents of non-contagious diseases will be most extensively applied as bacteriologic weapons. For this reason, utmost attention should be given to the defense of people who will be the direct object of the bacteriologic attack (2, 3, 4, 5, 6, 7).

It is considered that the most probable and effective method of application of bacteriologic warfare will be the aerogenic method, for which reason the defense against bacterial aerosols occupies one of the leading places in the A.B.D. system. Very serious attention is also given to the defense against the subversive methods of water and food contamination.

According to the official instructions published in the U.S.A., the antibacteriologic defense consists of: 1) detection and recognition of the bacteriologic weapon (indication); 2) individual defense of the population; 3) collective defense of the population and 4) decontaminating measures.

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## PRINCIPLES OF DETECTING THE BIOLOGICAL INFECTIOUS AGENTS.

The detection of biological infectious agents consists of a set of measures aimed at determining the fact that a bacteriologic weapon has been employed and at recognizing the kind of agent used.

Exact and timely ascertaining of the moment of employment of a bacteriologic weapon and the kind of agent used determines to a considerable degree the character and the specific direction of antiepidemic measures aiming at clearing up the consequences of the bacteriologic attack. This circumstance makes foreign specialists stress everywhere that the success of all the measures of antibacteriologic defense will depend largely on the perfection of detecting methods and on making the detection within the shortest time possible.

Methods of detection of biological infectious agents can be divided, according to their object, into two general groups:

- nonspecific detection methods (or physical detection methods) aimed only at establishing, by any signs, the fact that a bacteriologic weapon has been used;
- specific detection methods (or biological detection methods), aimed not only at discovery of bacteriologic attack, but also at recognizing the kind of agent used.

### NONSPECIFIC DETECTION METHODS.

The simplest, the fastest, but also the least

reliable method of nonspecific detection is visual observation. Bacteriologic attack can be suspected from the appearance, after ammunition explosion, of a cloud moving with the wind, from discovery in the site of explosion of powderlike substances or drops of liquid when no poisoning or radioactive substances are present in the place or in the air. Finding of unexploded special ammunition or its characteristic parts (remnants of atomizers, etc.), of masses of insects, ticks and instruments for their release (containers and other special carrier equipment) can also arouse suspicion of a bacteriologic weapon having been used.

Visual detection of a bacteriologic weapon is possible only through regular observation of all the territory of the country. However, as the authors of "Civil Defense Against Biological Warfare" (1) point out, the organization of such observation is at present impossible. Only the continuous observation of comparatively small, but most important, regions of the country is feasible.

Speaking of visual observation as a method of nonspecific detection we must stress that at its best it can provide basis for a supposition that a bacteriologic attack has taken place. In a number of cases, especially when the bacteriologic weapon is employed at night or in visibility limiting weather conditions (fog, etc.), visual observation can become an incompetent method of detection.

The extreme subjectivity of visual observation as a detection method dictates the necessity of critical evaluation of the data obtained through it and requires their careful verification by objective methods before reaching decision to carry out the measures of anti-bacteriologic defense, except the most basic ones (donning of gas-masks and protective clothing, using shelters).

An indispensable complement of visual observation is the determination of the quantity and extent of particles suspended in the air. The discovery of numerous airborne particles of determined dimensions following the appearance of enemy planes, bombing or artillery raid, permits drawing a much more definite conclusion than visual observation alone, regarding the employment of

bacteriologic weapon by the enemy.

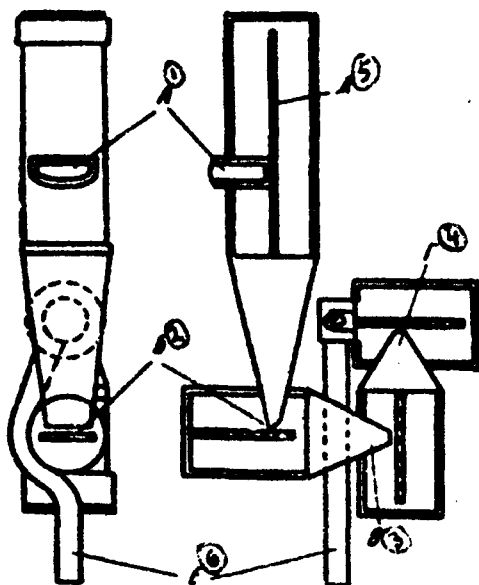
A whole series of instruments have been devised to date, permitting a rapid determination of the quantity and dimensions of airborne particles. Among them of particular interest are the impactors.

These instruments act on the principle of mechanical settling of aerosol particles on plates and their subsequent visual or microscopic examination.

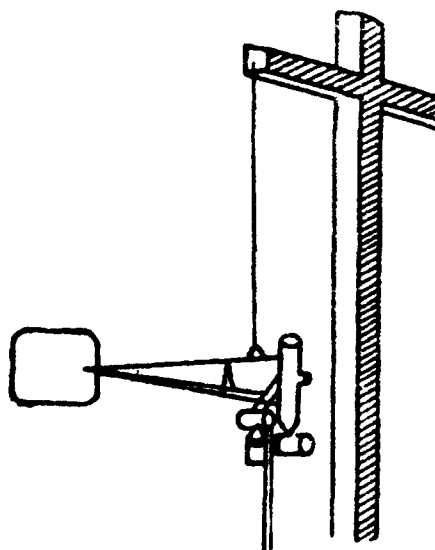
One of the first instruments based upon this principle was the impactor constructed by Hagemann (2), in which a circular air flow was directed towards an intercepting plate. The constructor, however, did not consider the necessity to observe the isokinetic conditions of the sample taking, so that considerable losses of large particles were noted during the operation.

Much more perfect is May's impactor (3), which has not one intercepting stage(cascade), but four (Fig. 12).

As can be seen from the figures presented, May's cascade impactor consists of a system of 4 jets and corresponding intercepting plates. The diameter of the jet nozzles decreases progressively from cascade to cascade, so that speed and, consequently, also effectiveness of the particle impact increases from plate to plate. The result is a sorting of particles according to their size, which greatly facilitates subsequent analysis of the samples. May's instrument is portable (weight 280 grams), reliable for work in field conditions and can be used for collecting any aerosol samples in which the dimension of the particles exceeds  $1\ \mu$ . For work in field conditions the instrument is suspended from a stand, as shown in Fig. 13.



**Fig . 12.** Diagram of May's  
4-cascade impactor.  
1-nozzle one; 2 -nozzle two;  
3-nozzle three; 4 -nozzle four;  
5-intercepting plate ; 6-pump  
pipe.



**Fig . 13.** May's impactor  
suspended.

Owing to a vane, the inlet of the instrument is always directed against the air flow. A portable injector, working on compressed air, is used for air suction. The optimal air suction volume is 17.5 liters per minute.

Preliminary treatment of the intercepting plates depends on the character of the aerosol particles to be investigated. For collection of samples containing solid particles, the plates of the impactor are covered with a sticky, non-drying film. Particularly effective is a mixture of castor oil and well purified tar.

Samples containing liquid particles are susceptible to evaporation; because of this, microscopic analysis should take place either before the process of evaporation has started, or a special kind of indicator must be used to preserve the drop form for a long time. The first method is applied only for the investigation of mists of oils or other low volatility liquids. For determination of the quantity and dimensions of the drops of rapidly evaporating liquids, the intercepting plates



can be treated with a mixture of light mineral oil and vaseline and covered with a layer of soot, (preferably of magnesium oxide - blackened over flaming magnesium shavings) and some other substances.

Analysis of samples is carried out under microscope with an ocular micrometer. However, as May points out, an experienced operator can easily determine the degree of dispersion of a sample, and consequently with even more ease, the change in quantity of particles in comparison with previous investigations, without the help of a microscope. Thus the presence of slight precipitation pattern on the first plate, some precipitation pattern on the second and thick precipitation on the third and fourth indicate that the aerosol under investigation is highly dispersive with predominance of particles from 1 to 10  $\mu$ .

The determination of quantity and dimensions of the particles can be effected by means of other instruments, working on different principles, especially electronic methods. Guyton (4) presents in his work two ways of using such methods. First of them is as follows: two electrostatic laminae are placed on the edges of the aerosol chamber, which in turn is placed under an ultra-microscope. After alternately charging the plates positively and negatively, the charged aerosol particles are set in a vibrating motion. Dimensions of the particles can be determined with considerable exactitude by photographing the vibrating motion.

Another method is based on the ionization of the particles by means of high voltage discharge. Charged particles are drawn towards metal plates having the opposite charge. At the same time a flow of air forces them aside. Since the smaller particles receive greater charge per mass unit, they settle with less displacement to the side from the ionizing electrode than larger particles. The side displacement of the particles is a mathematical function of their density and diameter and permits exact calculation of their dimensions.

A serious drawback of these instruments is their inability to determine, in addition to the dimensions, also the number of particles in the aerosol. This draw-

back is eliminated in the electrostatic counter, constructed at Camp Detrick by Guyton (4). This instrument works on the principle of electric pulses being produced by particles striking a copper filament.

The air being studied is sucked in at high speed through a vacuum pump nozzle toward the filament. The impact of the particles against the filament causes electric pulses in it. The electric circuit amplifies and fixes the pulses. The pulse is directed to a triatron tube for calculation, whose discharge is amplified in turn through a triode and induces a current in the electromagnet of a mechanical counter. The determination of particle size is based on prevalent dependence of the voltage on the square of particle diameter. The following formula is based on this relation:

Particle diameter (in microns)

pulse voltage (in microvolts)

3.04

The instrument used by the author could count about 2000 particles per second, which, however, was not nearly a final value. The smallest dry and liquid particles which this instrument was able to register were in the order of two to three microns, but according to Guyton's calculations, improvements in the construction of the instrument could lower this limit to 0.3-1  $\mu$ . Guyton's counter is portable and can be built into a medium-sized suitcase, which makes it easy to carry around.

Another instrument of a similar type is the photoelectric counter, constructed by Gucker, O'Konski, Pickard and Pitts (5, 6). Its action - to count the particles but not to determine their size - is based on light diffusion by aerosol particles. A narrow stream of the aerosol is intensely lighted against a dark background. The direct light diffusion observed during this operation is registered by means of a photoelement. The light diffused by each particle 0.6  $\mu$  or more in diameter, hitting the photoelement creates electric impulse which, after suitable amplification, becomes sufficiently strong to set off a mechanical counter. Particle counting progresses at the rate of 1000 readings per minute.

A general diagram of the instrument is presented on Fig. 14. This instrument was designed and first utilized as a penetrometer to test gas filters with the help of oil vapor, later however it was successfully used for the counting of particles in bacterial aerosols.

Recently this instrument has been improved by O'Konski (7). The new model has a higher counting speed and a differential pulse collector within the determined voltage limits. Introduction of the differential collector permitted to determine the dispersion of the aerosol under investigation.

By now, judging by the reports published in Army Information Digest (8) and Military Review (9), the work conducted at Camp Detrick has been concluded and a model aerosolscope has been constructed, which, according to the authors of the notice, evidently will find a wide application in nonspecific detection of biological infectious agents. We do not have data on the actual construction of this instrument, since its detailed description has not been given, however, we can

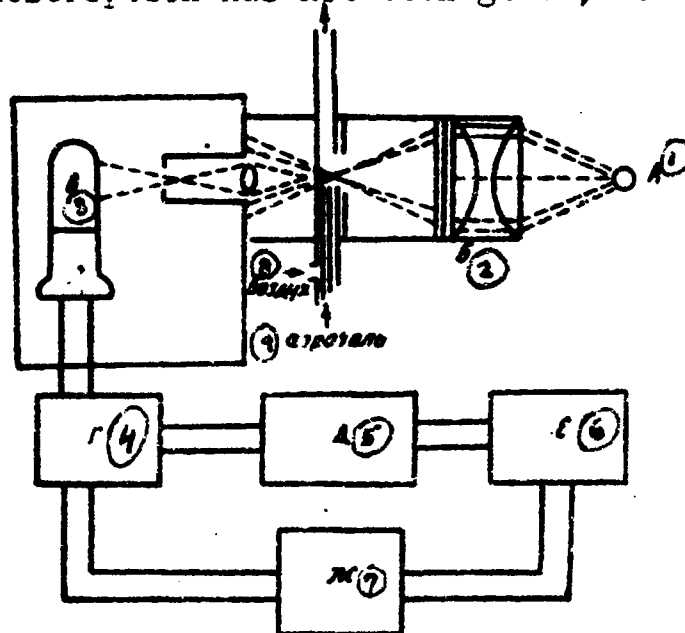


Fig. 14. Diagram of photoelectric particle counter.

1-lamp; 2-lenses; 3-photocell; 4 - amplifier;  
5 - cut-in circuit; 6 - registering and recording  
device; 7 - power source; 8 - air; 9 - aerosol.

assume that its action is based on one of the above described principles. The instrument counts the aerosol particles and determines their dimensions; it can also be used for the detection of not only biological agents, but radioactive particles as well.

The methods of nonspecific detection of bacterial contamination will evidently be used not only to discover bacteriologic attack by means of aerosols, but also to detect biological infectious agents introduced into the water works. As has been shown above, the American authors consider the water supply system as one of the effective ways of spreading pathogenic microorganisms and toxins (10). Diseases can be spread especially widely if biological infectious agents are introduced into the distributing system, from which water goes to the consumer without any purification. Their timely detection will permit shutting off water and thus averting the possibility of infection. Present measures of bacteriological safety of the water (coli-titer, microbial count) cannot give a timely warning signal, if only because of considerable lapse of time between the sample taking and obtainment of the results.

This indicates an urgent need for the development of nonspecific detection methods, which would permit immediate discovery of microorganisms or poisons in the water system. This last problem - rapid discovery of poisons - is very complex and there are no literature data on the possibility of its solution, but ways of a quick discovery of microorganisms in water have already been found. As shown by Berger and Stevenson (10), the solution of this problem was attempted by the workers of the Division of Physics at Camp Detrick. Results of the investigation have indicated that in principle this can be done by means of instruments with action based on the determination of light diffusion by bacterial particles (microphotometers); their exactitude, however, is very low and differs little from the exactness of general visual investigation.

In connection with this, the use of indirect method of detection of biological infectious agents in running water was considered to be promising. The gist of it is a constant control of residual chlorine concentration. For this purpose, installation of instruments in key positions of the water system is proposed, in order to assure an uninterrupted automatic determination

of residual chlorine content in the water passed on to the consumer. These instruments must be connected with a signal system and automatic equipment to collect samples for determination of bacterial contamination.

Although a drop in the water's residual chlorine content can be caused not only by the introduction of biological infectious agents, but by a series of other things (disturbance of the water-treatment process, unforeseen contamination of any part of the water system) as well, any of these reasons for the drop in chlorine concentration requires immediate discontinuation of the water supply, regardless of whether or not a bacteriological weapon has been used.

A direct microbial count in running water can also give definite results. An increase in the number of microbes will signal the danger of infection to the consumers. Collins and Kipling (11) developed a method of direct determination of microbial count in water, the application of which permits to obtain results 2 hours after sampling, i.e. much earlier than the generally accepted method involving inoculation on nutritional media. The essence of the method consists in coloring the microorganisms contained in the water with 0.1% solution of gentian violet in a mixture of distilled water and glycerine, and subsequent concentration of the microbes through water evaporation. The resulting concentrate, a suspension of microorganisms in glycerine, is pumped into capillary tubes of determined diameter, in which the count is effected under a microscope.

The increase of bacterial concentration in the air and running water can be determined not only through count of the particles suspended in them, but also by determining the protein concentration, since protein discovered in the air is in the majority of cases genetically connected with microorganisms.

Stickland's work (12) shows the possibility of application of biuret reaction to determine the number of bacteria. The essence of his proposed method boils down to the addition of sodium hydroxide and copper sulfate to a bacterial suspension. The intensity of the purple color appearing as the result and measured by means of photoelectroscopic absorption meter, is proportional to the number of bacteria. Certain groups of organisms, particularly staphylococci, require preliminary

heating of the suspension with sodium hydroxide, at 100° for a few minutes, with subsequent addition of copper sulfate.

The drawback of Stickland's method is its insufficient sensitivity. Optimal quantity of protein determinable by it is 0.5 mg., which corresponds approximately to 5 mg. of dried microbial bodies. Lesser protein content in the sample leads to still lower exactitude of the method.

This exactitude can be increased through the introduction of certain changes (13).

American journal "Science News Letter" published (1958) a report on the possibility of applying the pyrolytic reaction to determine the presence of bacterial protein in the air. The method is based on the fact that when the microorganisms collected from the air are heated to 315-482°, protein decomposition is caused, and one of the products obtained is prussic acid, which is easily determinable by a number of methods. This procedure, developed on contract for the Army at Southern Scientific Investigation Institute in Birmingham (Alabama), is simple, fast and highly sensitive. It permits to discover as little as a few micrograms of bacterial protein in a liter of air.

#### SPECIAL DETECTION METHODS.

Special detection of biological infectious agents consists of three basic stages:

- collection of samples;
- accumulation of the agents present in the samples under analysis;
- separation and identification of the microorganisms present in the sample.

##### Collection of samples.

Samples are collected in the region of presumed site of employment of the bacteriological weapon. The collection has two objects: supply of material to the laboratory for isolation of the agent used and recognition

and limitation of the infected zones.

The following are investigated: air, water, food, washings from different kinds of surfaces, fragments of aerial bombs and instruments, spraying apparatus used and various kinds of smaller objects (such as leaves, stones, fragments of instruments, etc.).

During sample collection it must be remembered that laboratory analysis is a complex and laborious process; consequently, samples should be taken very selectively, taking into consideration the maximal probability of isolating an agent and always limiting the number of samples submitted to the laboratory.

The problem of a best method for air sample collection has not yet been settled.

Abroad, a whole series of instruments has been created to date, none of which, however, can be considered fully satisfactory. This is proven by the fact that in special American instructions on bacteriologic weapon defense, the most varied devices, down to the simplest, are recommended for sample collecting. According to their action, the instruments currently used for collection of air samples can be divided into two basic types, as follows:

- sedimentation;
- aspiration.

Large number of instruments proposed for collection of air samples makes detailed description of each model impossible and limits the review only to those recommended for use by foreign, and especially American, instructions on bacteriologic weapons defense, or those developed at scientific centers conducting studies on bacteriologic weapons (Camp Detrick, Porton) and in institutions connected with them by contracts.

The method of bacterial air pollution investigation by means of a Petri dish with solid nutritional medium, which is an example of a sedimentative device, apparently has not yet lost its significance, in spite of its simplicity and grossly insufficient exactitude. This is borne out by the fact that in "State of Maine Medical Civil Defense Plan" (15), set forth as a model for the organization of similar plans in other states, it is specified that Petri dishes be included among the equipment of Civil Defense Corps. Open dishes are to be placed

for a definite period of time both on the roofs of high buildings and on the ground.

After such exposition, the dishes are shipped to a suitable laboratory for incubation and further investigation.

This method, however, has among a whole series of drawbacks the most essential one, which makes doubtful the possibility of its use for bacteriologic weapon detection. It is a fact that by means of the plate method only those microorganisms which are attached to the largest aerosol particles can be intercepted; this makes it unsuitable for the investigation of fine-dispersion aerosols.

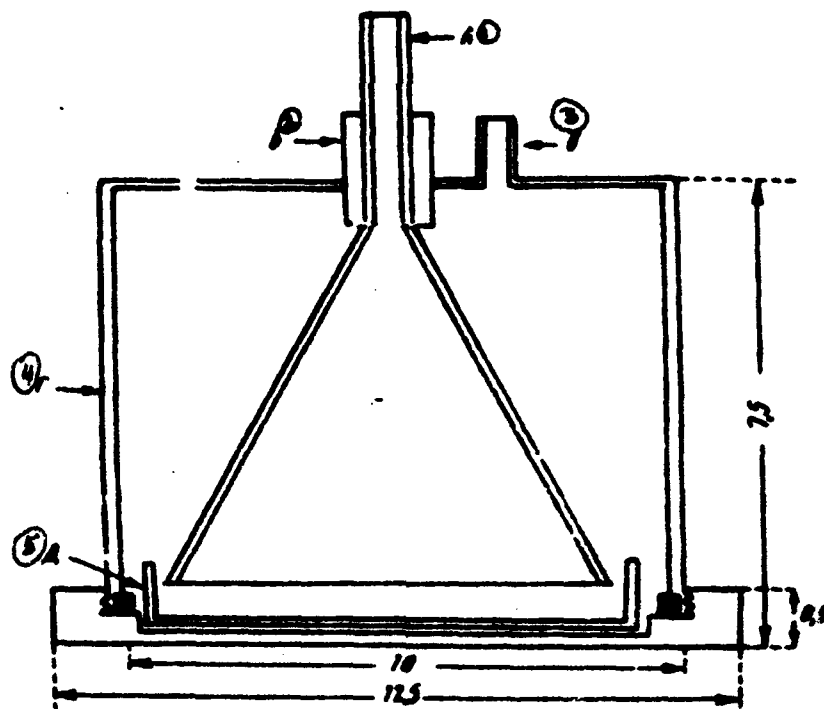
Among the instruments recommended for use is the one proposed by Hollaender and Della Valle, back in 1939. Its action is based on the principle of collision of the particles with the surface of agar. The instrument, basic diagram of which is shown on ill. 15, is simple and easy to handle.

Air samples are collected by means of an ordinary pump, connected to an air flow speedometer. Recently, the Petri dish with solid nutritional medium has been replaced in the Hollaender and Della Valle instrument by special filters, impregnated with nutritional medium, proposed by the U.S. Health Service.

Du Buy and Cripps (17) instrument with a screen disc has also been recommended for use. As in many other devices, Petri dish is used for particle interception. Air flow is directed towards the surface of the agar through perforations in the disc, which is located close to the said surface.

The instrument consists of a round body with an air outlet, a Petri dish and a brass cover-disc with 300 perforations. The cover snugly closes the body, to which it is attached by means of two surface clamps. The air enters the instrument through the perforation in the cover disc, hits the surface of agar, then glides on the medium surface and is forced out through the outlet at the bottom of the case.





**Fig. 15.** Hollaender and Della Valle instrument for collection of air samples.

1-glass funnel; 2-rubber plug; 3-pump flue;  
4-body of the instrument; 5-Petri dish.

In the U.S. Army Chemical Corps laboratory, Andersen (18) constructed a more perfect variation of the Du Buy and Cripps instrument. This model distinguishes itself by a greater intercepting effectiveness because of the cascade impactor principle used in it. The instrument is composed of 6 elements, one placed above the other. Each element consists of a Petri dish with agar, over which a perforated disc is placed. The diameter of the perforations decreases from the top disc to the bottom one, which results in increasing speed of the air flow from one cascade to another and, along with it, increasing impact effectiveness - larger particles settle on the top plates, while smaller do so on the bottom ones. Thus Andersen's instrument not only assures a more effective interception of the aerosol particles, but also permits to determine which aerosol fractions carry the pathogenic microorganisms. Diagram of the instrument and its general appearance are presented on Fig. 16 and 17.

Various models of "slot" instruments can also be used for collection of air samples. One of the first models of this kind was a "slot" instrument for collecting air samples and counting bacteria, proposed as early as in 1941 by Bourdillon, Lidwell and Thoma (19), built in the form of a cylinder, covered at the top with a lid, provided with a V-shaped slot. A Petri dish with medium is placed under the cover. An electric motor draws in air through the cover slot and assures a uniform rotation of the plate. Passing through the slot, the air hits with a great force the surface of the medium and inseminates it with microflora.

Later Bourdillon, Lidwell and Shuster (20) perfected the "slot" instruments by equipping it with a device to register the temporary changes of microbial concentration in the air being investigated.

Shuster (21) constructed a "slot" instrument which takes samples automatically.

In recent years this instrument has undergone further perfection, which resulted in the construction of a portable model capable of protracted air sample taking (22).

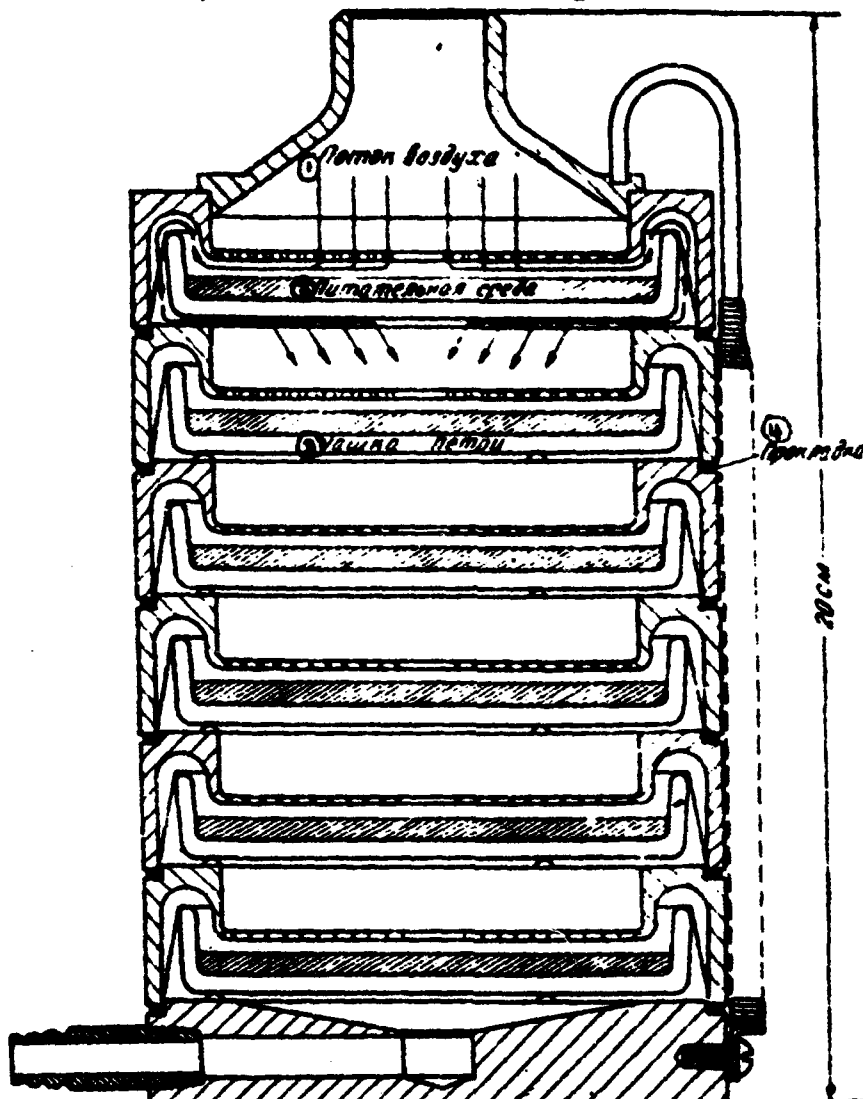
It can take air samples for 12 hours at a speed of 28.3 l. per minute. A study of its effectiveness in taking samples of bacterial aerosols was conducted by Kuehne & Decker (23) at Camp Detrick and showed that it possessed numerous advantages over the standard models in existence. Its particular value is that it permits to carry out air analyses over a long period of time and provides constantly a high effectiveness at a minimal input of work.

Air samples can also be taken by instruments of the Wells' centrifuge type (24). Wells' air centrifuge consists of a glass container, electric motor and a case. A stream of air is sucked into the opening of a rapidly turning



Fig.16. General view of Andersen's instrument for air sample collections.

cylinder. The cylinder contains a glass test tube, the



**Fig. 17.** Diagram of Andersen's instrument.  
1-Air flow; 2-Nutrient medium; 3-Petri dish; 4-Gasket inside surface of which is covered with a layer of solid nutritional medium. The centrifugal action causes the particles to settle on the surface of the medium.

The action of aspirative type instruments for the collection of air samples is based on the principle of electrical or thermal settlement of aerosol particles.

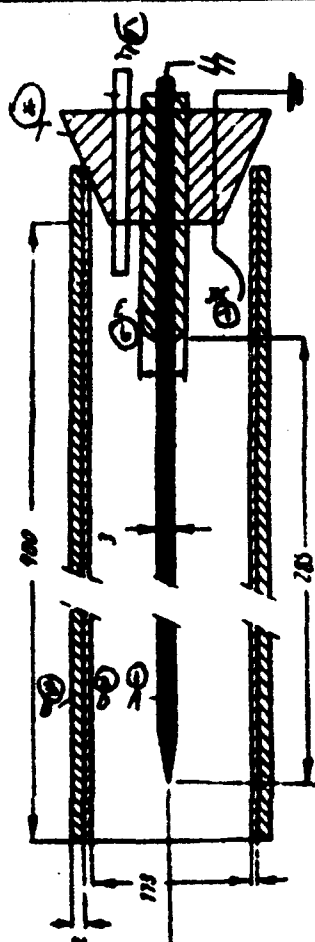


Fig.18. Diagram of collector tube of electrostatic precipitator with agar.  
1-high voltage electrode; 2-grounded agar layer; 3-glass tube; 4-rubber stopper; 5 -opening for air exhaust; 6-contact on agar; 7-grounding contact.



Fig 19. Agar collector tube with *B. prodigiosum* colonies after their electrostatic settlement at 10 kV.

The principle of precipitation in electrostatic field is particularly put to work in the electroprecipitator constructed by Luckiesch, Taylor and Halladay (25). In this instrument two Petri dishes are used, in which the medium is placed directly on the electrode plates. Precipitation of particles on the medium's surface takes place as an electrostatic field is produced on the electrodes when 8000 volts are supplied.

Another instrument working on the same principle

was proposed by Maissonnet (26).

Houwink and Rolvink (27) constructed two new models of electroprecipitators. The first of them is an instrument, the main part of which is a glass tube, covered inside with a layer of nutritional agar. A high tension electrode is fastened in the center of the cylindrical collector. The agar layer is grounded, while positive charge is given to the central electrode (Fig. 18). The microorganisms are sucked from the air through the tube and precipitate on agar covering the walls of the glass tube. The tubes are then placed in incubator for subsequent culturing. Fig. 20 shows an agar tube with *B. prodigiosum* colonies after electrostatic precipitation and subsequent incubation.

The second model of precipitator has a water-film collector, permitting a prolonged admission of aerosol samples. Diagram of this collector is presented on Fig. 20.

A film of water flowing down the inside walls of a vertical cylindrical tube is the negative electrode. A lead in the center of the same tube is the positive electrode of high voltage. Particles are caught in the liquid, which is subsequently analyzed.

Along with the electroprecipitators, thermoprecipitators can be used, in which precipitation of the particles occurs as the result of temperature differences between the walls of a chamber through which passes the aerosol under investigation. In the recent years, a group of scientists (28, 29, 30) at Georgia State Institute of Technology, working under contract with the U.S. Army Chemical corps, carried out the construction of thermoprecipitators and investigated their effectiveness. As a result, a model instrument was constructed, serial production of which has been undertaken by a manufacturing company. The precipitator constructed by the above authors has three basic parts: a lower cooled plate, an isolating layer and an upper heated plate. Heat is supplied by the usual electrical elements, and the cooling effect is obtained through the circulation of running water. For investigation of bacterial aerosols the temperature gradient should not exceed  $100^{\circ}$  (heating to  $125^{\circ}$ , cooling to  $25^{\circ}$ ). The air is pumped through an inlet in the center of the upper plate and passed in radial streams between the heated and cooled surfaces, the distance between which is 0.038 cm. Any particle contained in the air passing through the thermal field settles on the cooled surface

or on the substrate covering it. As substrate the authors used filter paper impregnated with a nutritional medium (agar with various additives) on which culturing was then continued.

In many instruments for collection of air samples the microorganisms are intercepted by means of various liquids in form of foam, bubbles or drop aerosols. The first is used in the instruments developed by Robertson, Brigg, Miller, Baker (31), Wheller, Foly, Jones (32), Lemon (33), which are variations of the well known instrument of D'yakonov and of the "capillary impinger" of Rosebury (34) and many others. Moulton's instrument makes use of the principle of interception of the investigated aerosol particles with minute drops of liquid medium, dispersed by a flow of pumped in air.

Instruments using liquids for purposes of interception of the air microorganisms give much more accurate results than the ones using solid nutritional media or membrane filter. The main reason for it is that on solid nutritional medium an aerosol particle originates only one colony, no matter how many microorganisms are in it. In the instruments using liquid, however, division of conglomerates takes place.

Together with this very important advantage, which is more valuable for investigational work than for detection, the atomizers possess a substantial drawback, which greatly limits the possibility of their use outside the framework of experimental investigations. The majority of proposed models of atomizers are not meant to process great volumes of air. Thus Moulton's atomizer can process not more than 4.5 l. of air during 10-15 minutes, while Rosebury's "capillary

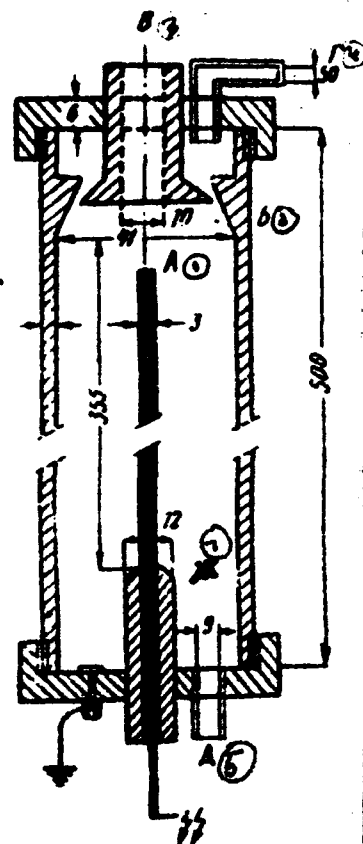


Fig 20. Diagram of collector tube of electrostatic precipitator with water-film.  
1-high voltage electrode; 2-collector tube; 3-air supply; 4-water supply; 5-air and water outlet; 6-casing for the water-film formation; 7-electrode insulator.

impinger" can handle 2-3 l. per minute. Robertson's instrument is somewhat more productive (5-10 l. per minute). The U.S. Health Service developed it into a twelve-flask sample collector, which is planned for use by the Civil Defense Service. The twelve-flask collector is built in such a way as to assure a continuity of sample taking. Through each flask, filled with nutritional medium, air is pumped for 20 minutes; thus it can take samples for 4 hours without interruption and without recharge.

Judging by extant data it seems that the instruments in which the most varied filtering materials are used for interception of the microorganisms, will find a wide application. A simpler instrument of this type is the "cotton impinger", which is described, and instruction for its use given, in the work of Hantover (35). "Cotton" impinger is very simple and can be used in any conditions. It has the form of a truncated glass cone containing cotton balls. A hand pump is attached to its narrow end, and a small glass tube is inserted into its wide end. Metal net is placed before the cotton balls for a more uniform distribution of particles over the cotton.

After collection of the air samples the instrument is placed in a protective case and sent to the laboratory, where the particles caught on the cotton are eluted and culture made with the suspension obtained.

Membrane filters can also be used for isolation of microorganisms from the air; their application is recommended by many special manuals. Continuous sample taking instruments have been constructed using membrane filters as interceptive material. Detailed description of one model of a similar instrument is given in the work of Crist, Gurney & Hansen (36). The instrument contains several filter holders, connected to an air pump. A special device opens, at the proper time, a filter holder valve, thus assuring the pumping of the necessary quantity of air through the filter, and then closes it, directing the air towards the next filter.

For investigation of aerosols special membrane filters are used; they differ from the filters used for hydrosols in that their pores are larger.

There is a great diversity of opinion concerning the performance of nitrocellulose membrane filters in intercepting microorganisms from the air. Some authors say

the method is "ideal", others (27) point out that membrane filters tried by them gave completely unsatisfactory results in the investigation of aerosols for vegetative forms of microorganisms, since among the intercepted cells the number of viable ones did not exceed 10%.

Recently filters made of soluble materials, especially dry gelatine, gained wide acceptance. The technic of preparation of these filters was worked out by Mitchell, Timmons and Dorris (37). The foam for the filters is prepared from 40% gelatine solution in distilled water, containing 4% glycerine. This solution is poured into rectangular forms made of wax paper, which are then placed in vacuum dessicator with calcium chloride. The air is extracted from the dessicator by means of a pump, until the disappearance of all bubbles and formation of a foam layer. Drying under vacuum is continued for 3 days. Towards the end of this period, the gelatine foam dries, becomes porous and sufficiently stretch-resistant. In this form it is suitable for preparation of the filters. At present, foam gelatine is industrially manufactured by an American company.

The size of the pores can be controlled by varying the thickness of the gelatine layer at the time of pouring it into the forms. Thin layer has smaller pores than a thick one.

The process of preparation of the filter out of a gelatine block is described in detail in the work of Mitchell, Fulton and Ellingson (38). The filters are cut out of the blocks with the help of special pattern with a cutting edge and a circular knife (Fig. 21).

The form and dimensions of a ready filter are presented on Fig. 22.

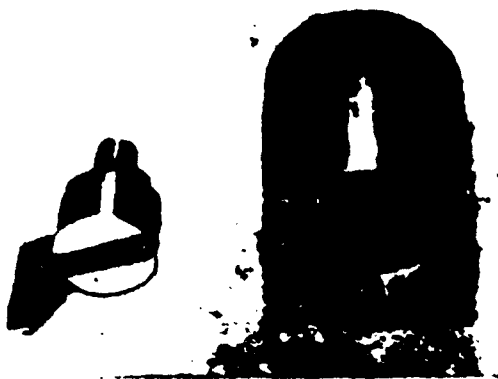
Filters are sterilized with a mixture of ethylene oxide and carbon dioxide ("carboxide"). However, certain authors point out that this treatment of the gelatine filters with ethylene oxide lowers their solubility, and for this reason they suggest that non-sterile filters should be used, disregarding the insignificant number of microorganisms that could invade them during preparation (39).

Finished filters are placed in a paper envelope, both sides of which have circular openings in the center; the convex part of the filter fits into one of the openings. To protect them from impurities, the filter



is packed, together with the envelope, into several wraps (Fig. 23).

**Fig. 24** shows the instrument for collection of air samples, in which a foam gelatine filter is used. It consists of a suction tube with rheometer, a filter holder and a closing device-sample taking tube, connected with the source of negative pressure.

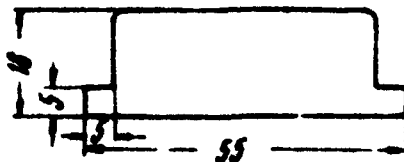


**Fig. 21.** Knives for cutting out filters from gelatine blocks.

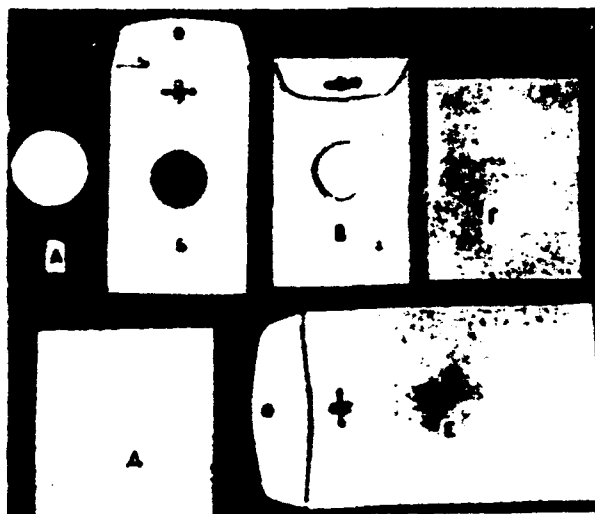
Before taking an air sample, the filter, recently unwrapped from all the protective wrappers (except the envelope-container), is inserted into the filter-holder and pressed snugly in with the lower, mobile part towards the higher, immobile one. After the air has been sucked through, the filter is removed, placed in a sterile envelope and sent to the laboratory. Before examination, the filter is dissolved and the microorganisms contained therein suspended in water.

Comparative investigation of performance of the gelatine filters, membrane filters and certain interceptors of bacteria, carried out by Mitchell, Fulton and Ellingson (38) showed that gelatine filters have the best intercepting ability. They also have many further advantages. In particular, they can be successfully used for collection of air samples at low temperatures, when the instruments using liquid media or media with agar for interception of microorganisms are useless because they freeze. Also, any instrument requiring nutritional medium is not very suitable for use under field conditions, since it is very difficult to protect nutritional broth or agar from contamination during transport.

Another material which can be used for the preparation of soluble bacterial filters is sodium alginate, which is a fibrous material, similar to cotton in appearance. It is easily soluble in water (down to 1 part per 10 parts of water) and tolerates sterilization at 125°.



**Fig. 22.** Gelatin filter;  
actual size.



**Fig. 23.** Packing of gelatin  
filters (letters indicate the  
sequence of the wrappings).

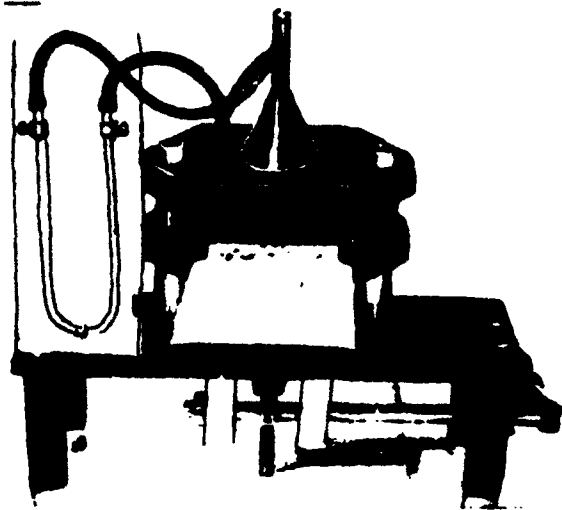


Fig. 24. Instrument for collection of air samples, using gelatine filters.

The instrument presented by Richards (40), in which sodium alginate is used as interceptive material, is both simple and easy to operate. It has the form of a metal tube 75 mm. long and 12 mm. in diameter. One end of the tube is open, the other - joined to a pump. Inside the tube is a plug made of sodium alginate fibers. After the necessary quantity of air has been drawn through it, the plug is placed in sterile water, which after the complete dissolution of sodium alginate undergoes further investigation. Checking of the effectiveness of these filters always showed the air that passed through them to be sterile; i.e. all airborne microorganisms remained on the filter.

Among the materials suitable for preparation of soluble filters, we should mention also sodium glutamate, recommended by Japanese authors (41).

Concluding the review of instruments and methods for sample collection, we must note that their division into those provided for non-specific detection and those for specific detection, as made at the presentation of the material, is doubly conditional. Certain instruments meant for determination of number and size of aerosol particles, can also be successfully used for collection of samples destined for bacteriologic investigation in order to determine the genus of the microorganisms contained in the

air. Thus Sonkin (42) successfully used May's cascade impactor for collection of air samples in order to isolate streptococci and pneumococci from the air. The method he used permitted not only to count particles in aerosols and determine their size, but also to culture the microorganisms which settled on the intercepting plates of the impactor.

Also, appearance of an unusually great number of colonies on Petri dishes, inoculated by means of "slot", screen disc, or similar instruments, or on the membrane filters through which the air under investigation is drawn, permits to suspect that a bacteriologic attack is under way even before the identification of pathogenic microorganisms. Evidently, American scientists value highly this characteristic. In connection with the fact that during systematic investigation of air the laboratory personnel will have to count colonies on a great number of dishes, recently attempts have been made to construct instruments which would permit to speed up greatly this process. Such investigations were especially carried out, on assignment from U.S. Chemical Corps, at Du Mont laboratories. The construction of such an instrument was presented by Mansberg (43). It is based on the principle of television camera action. General appearance of this instrument is shown in Fig. 25.

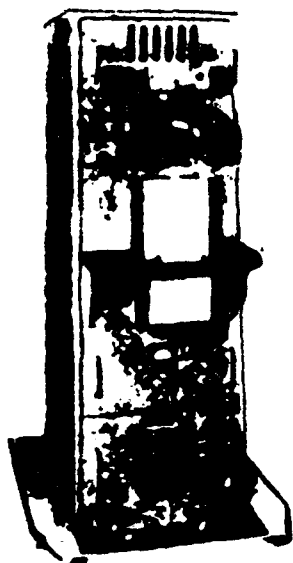


Fig. 25. General appearance of the instruments for counting colonies on plates.

Petri dishes with developed colonies are placed in a counting chamber, where a cathode tube and an optical system are used to divide their surface into strips.

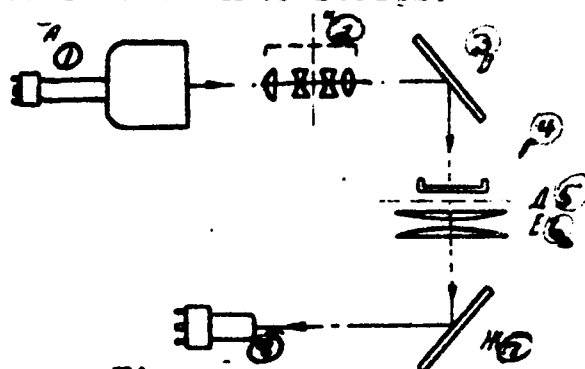


Fig. 26. Diagram of the optical section of the instrument for counting colonies on plates. 1-electron beam tube; 2-objective lens; 3-mirror;

4 -searching device; 5 -screen;  
6 -condenser lenses; 7 -mirror;  
8 -photoelectric multiplier.

26. A diagram of the optical system is presented in Fig.

The analyzing light spot produced by the cathode lamp is directed by means of the objective lenses to the surface of nutritional medium. Light from this moving spot, passing through the thickness of the nutritional medium and the plate walls, is intercepted by the condensing lenses and forms an illuminated segment on the photocathode of the intensifying photocell. Every time the analyzing light spot is darkened by a non-transparent or semi-transparent colony, the photocell produces a pulse. When a great number of the analyzing strips are used, the number of pulses will be proportional to the number of colonies and their sizes. If the diameter of all the colonies were almost equal, then, starting with the intercepted number of pulses and their duration, it would be very easy to determine the number of colonies. However, the size of these colonies can vary considerably, and thus, when the surface of the medium is divided into strips, the ray can hit the same colony several times, and this colony can originate not one, but several pulses. To avoid counting the same colony more than once, the instrument has a special "remembering device", which excludes this possibility. The "remembering device" acts in such a way that reading does occur only when the light spot hits the colony for the first time; when it happens again, the pulse is weakened and extinguished before reaching the reader.

The instrument for automatic colony counting has been successfully tried out in many laboratories. To count the colonies on one Petri dish, not more than 2 seconds are necessary. Under ideal conditions the count is 100% accurate; under usual conditions it is somewhat less so, but still not inferior to the visual method.

A modified model of the instrument permits counting colonies growing on membrane filters.

As we have mentioned before, upon application of a bacteriological weapon, not only air can be the source

of infection for man, but also water, soil and other outside objects. This circumstance makes it necessary to investigate samples of water, earth, foodstuffs and other objects, for presence of biological infectious agents. The great attention devoted in this work to the collection of air samples does not in any way imply lesser importance of investigation of other objects for purposes of detection, but only the greater complexity of air sampling and an insufficient development of this problem.

In reality, the investigation of earth, water and other samples can in many cases give much more accurate indications that a bacteriological weapon has been used. This is connected with the fact that catching volatile aerosol, especially during high speeds of air streams, is quite a complicated task. It is much simpler to detect the microorganisms settled on the soil, tree leaves, walls of buildings and other objects.

The necessity for water investigation is dictated by the fact that water supply sources serve themselves as an object of bacteriologic attack. The methods of non-specific detection of the biological infectious agents in water system has already been briefly mentioned above.

Collection of water samples for isolation and subsequent identification of the microorganisms used as bacteriologic weapon does not significantly differ from the generally used methods.

The State of Maine Defense Plan (15) contains the following instructions on the collection of water samples:

1. From a tap of undamaged water system.

a) Turn the tap on and let the first portions of the water run off.

b) Remove the closure from a standard container (if none is available, a jar with screw-on top may be used); place the container under the tap without touching the edge, fill it with water almost to the brim, put on the lid and wrap it up in a piece of fabric. If a jar is used, put on a gasket before screwing the lid on.

2. Taking water samples from rivers, ponds and reservoirs.

a) Before sample taking, wash hands thoroughly with soap and water to remove bacteria.

b) Take off the fabric cover and remove the stopper from the container for transportation of the material.

c) Put hand as far out as possible, downstream, immerse bottle about 40 cm. deep in water, with the mouth against the stream and fill it with water.

d) Close the bottle.

The instructions for taking water samples from wells, springs and similar sources are analogous.

Acquaintance with them shows that the methods and equipment for collection of water samples are the same as those generally used for microbiologic analyses. It only should be pointed out that the already cited work of Berger and Stevenson (10) mentions the instruments to be installed in the water system, for automatic collection of samples. These instruments are connected with the apparatus for non-specific detection of biological agents in the water system and work upon receiving signal from it.

It seems that membrane filters will be widely used for purposes of concentration of microorganisms in water samples. Recently appeared reports state, however, that diatomite filters (diatomite suspension on cotton tissue) are more effective than the membrane ones. According to Lyutov's data (14), they have much better filtering ability. A diatomite filter can process up to 100 l. of water in 35 minutes. The author was able to detect *Salmonellae* in water present there in very small numbers (5-6 organisms per 25 liters).

When a bacteriologic attack is suspected to have occurred, it is also necessary to take samples from the soil, especially in the place of explosion, from tree leaves and washings from buildings, road surfaces and clothing of people who got into the way of an aerosol cloud.

As shown by American special instructions, sample taking from surfaces has two objects: to identify the kind of agent employed and to define the infected area. Relatively smooth, clean surfaces such as metal, glass, concrete, asphalt or finished wood are best for sample taking. Samples should be taken from the surfaces perpendicular to the air stream. If the direction of the wind is unknown, samples should be taken from all 4 possible directions. Taking samples from horizontal surfaces is also permissible; moreover, in this case the direction of the air movement does not have a

great influence.

Sample taking from the outside surfaces should be accompanied by making corresponding notations on the map (45). This not only facilitates subsequent marking of the infected regions, but also simplifies the work of laboratories, which can refuse to analyze specimens collected too far from the infected places.

Inside buildings samples should be taken both from horizontal and vertical surfaces. If there is air conditioning, it is sufficient to collect samples from the surfaces in the proximity of the "in" and "out" openings.

The method of sample collection from surfaces is relatively simple: it consists of wiping them with a moist cotton wad. In place of these, some authors (46) propose the use of tampons of soluble materials, especially calcium alginate, which assure a higher percentage of microbial restoration.

Along with the surface samples Hantover (35) recommends taking, for investigational purposes, smears from nostrils, throats, ears, mouths, conjunctival cavity and skin of people in suspected areas. According to Hantover, experiments have shown that the most accurate results are produced by smears from the oral cavity.

Various objects (leaves, stones, munition fragments, remnants of atomizers) as well as small dead animals and carriers from the region supposedly attacked by bacteriologic weapons, should also be sent to laboratory for investigation.

The U.S. Army has special kits for the collection of samples in field conditions, provided for taking samples of air, water and other objects which can be infected (45).

An air sample is collected by means of drawing the air with a hand pump through the impinger, filled with special intercepting liquid, included in the kit.

Later the sorptive liquid is passed through a membrane filter, which undergoes further investigation.

For collection of samples from surfaces, special packaged tampons are included in the kit. A sample is taken by rubbing the surface suspected of infection with moist tampon.



To eluate the infectious agents from the tampons, gelatin solution is used. The eluate is passed through a membrane filter.

The kit also contains small polyethylene bags, in which to put the different objects to be sent to laboratory (ammunition fragments, leaves, stones, small dead animals, etc.).

The used membrane filters are sent to the laboratory in a special hermetically sealed box, inside which they are placed on nutritional medium. Boxes containing the filters are then placed in a waist-incubator, the description of which follows below.

A general view of the kit is presented in Fig. 27, 28.

#### Rapid accumulation of microorganisms in samples.

After the collection of samples, the next step of specific detection of biological infectious agents is the accumulation (growing) of the microorganism contained in them and the isolation of a pure culture.

As is well known, the process of growing microorganisms, especially viruses and rickettsiae, requires considerable time. For this reason investigators working in this field have made attempts to shorten as much as possible the time necessary for the growth. Up to date, several ways to solve this problem have been found.

The first of them consists in a maximal cutting down of the time elapsed between the moment of collection of the sample and the start of incubation. In case of bacteria, this can be achieved by immediately placing the collected sample in the thermal conditions in which the intercepted microorganisms start to develop already during the transport to laboratory. American instructions advise the warmth of the human body for this purpose. Thus, filters used in the apparatus of Hollaender and Della Valle, according to the instructions given in the already frequently mentioned Defense Plan of the State of Maine, should be placed on special pads saturated with the medium, wrapped up in polyfilm and put in an inside pocket of the man transporting the filters to the laboratory.

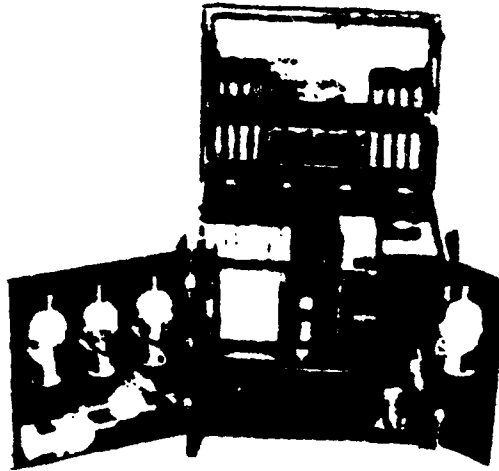


Fig 27. Field kit for collection of samples.

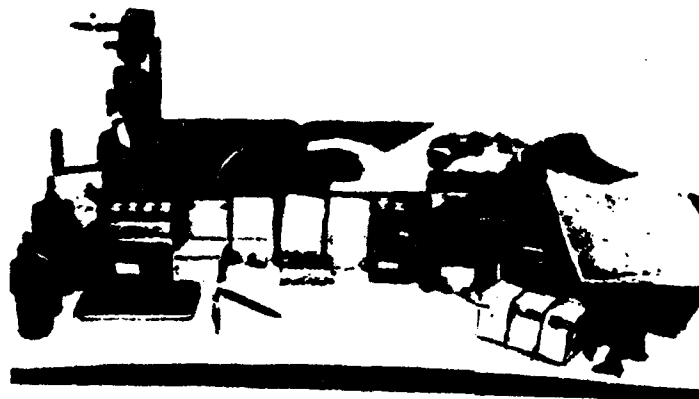


Fig 28. Field kit for collection of samples.

Laubusch(47, 48) proposed a special vest (Fig. 29) for the cultivation of microorganisms on membrane filters, using the warmth of a human body. The used membrane filter is placed on a pad saturated with medium and then in a flat box, hermetically sealed. The boxes are carried in one of the inside pockets of the vest.

To speed up the growth process of the investigated microorganisms developing on an artificial medium, the method of culturing them in continuously changing nutritional medium can be used.

This method assures uninterrupted removal of the products of microbial metabolism, thus speeding up the microbes' growth.

Harris and Powell (49) of the British Scientific-Investigational Center in Porton, proposed the construction of a special chamber, which would permit continuous change of the nutritional medium. Using this chamber the authors were able, according to their own information, to shorten the whole process of identification of the isolated microorganisms (inoculation, growth, morphological study, reaction with immune sera) to 5-6 hours.



Fig. 29. Vest incubator.

The chamber operates on the principle of culturing microorganisms on the surface of cellophane membrane, under which the nutritional medium is continuously exchanged. The chamber is mounted on a metallurgic microscope, due to which the observation is carried out in reflected light.

Fig. 30 shows a basic diagram of the chamber. Its body has the form of a stainless steel cylinder, with a depression on one side (1.5 mm.). Cellophane is pulled over this depression and fastened by means of a washer and a screw-on clamp. Two openings connect the depression with the tubes supplying and removing nutritional medium. To decrease the dispersion of light hindering observation, the bottom of the depression is covered with dark, dull enamel.

The body of the chamber is placed in an aluminum container (Fig. 31), which in turn is fastened on a microscope stage.

To supply air to the cellophane membrane, a small groove on the upper surface of the holder is connected by a tube, built into its body, with a small air pump or a cylinder with compressed air or other gas (e. g. nitrogen, indispensable for anaerobic cultures), (Fig. 32).

The top of the chamber is closed with a screw-on aluminum lid, equipped with an opening for the eyepiece of the microscope. Assembled instrument is shown in Fig. 33.

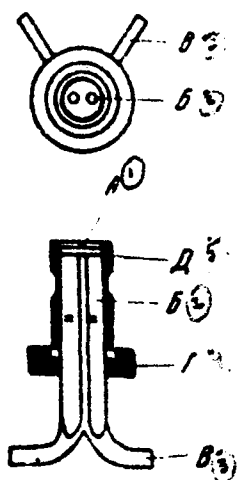


Fig. 30. Cross-section of the chamber.

Top-view from above. Bottom—a cross-section. 1-cellophane; 2-opening for supply of nutritional medium; 3-tubes for supply of nutritional medium; 4-connector; 5-screw on clamp.

The chamber, together with the microscope, is placed in a room at 35-37°.

To assure continuous supply of the nutritional medium under the cellophane membrane, the authors proposed a special device, made of glass (Fig. 34). It works as follows: the reservoir B is half filled with medium and plugged with cotton. Filtered air slowly passes through tube C, raises in bubbles up sleeve D and goes out through the stopper. The medium, raised up by the air bubbles and carried into the reservoir, assures the passage of a weak stream of medium through tubes A and A<sub>1</sub> into the chamber. Valve E allows for exchange of the medium. Air supply is regulated by a clamp or valve at the entrance of tube C.

Kanz constructed a similar instrument for culturing microorganisms in continuously changing nutritional medium. It is based on a Petri dish with cellophane pulled over it and a device assuring the circulation of the medium.

The third direction taken by the work on discovering ways and methods of speeding up the microbial growth process is the preparation of nutritional media, causing a more vigorous development of the microorganisms. A number of reports proves the possibility of creating such

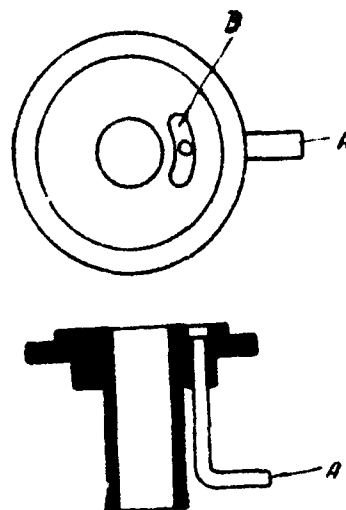
media by means of addition of various kinds of substances stimulating growth and multiplication of the microorganisms. Girard and Gallut (51) showed that the addition of filtrates of liquid *Pasteurella pestis* cultures to nutritional broth not only considerably speeds up the growth of this microbe, but also makes it possible to use a lower inoculation dose. On the basis of the experiments effected, the authors recommend the use of media with culture filtrates in order to speed up bacteriologic diagnosis.

Corn extract has also been suggested as a stimulant of growth in certain microorganisms (52, 53, 54).

Certain other substances also possess a pronounced stimulating action. Japanese authors show stimulating action of bamboo shoots (55) and bone marrow of bovines (56). Hara (57, 58) observed that passing electric current through a medium increases the growth of the microorganisms. This manifestation, according to the author's data, is connected with the accumulation of small quantities of  $\text{NaClO}$  in the medium under the influence of the current. This substance produces a pronounced therapeutic effect.

#### Identification methods.

Cultivation of the microorganisms found in a sample is not an indispensable stage in the process of their identification. Application of certain methods permits to determine the kind of microorganism directly in the infected material, which shortens significantly the time necessary for identification.



**Fig. 31.** Chamber holder.  
A—gas supply tube;  
B—groove for gas distribution.

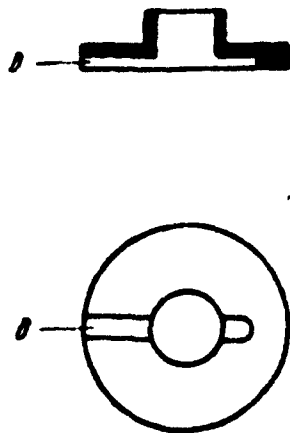


Fig. 32. Chamber cover. Top: cross section. Bottom: view from below.  
B-groove for direction of gas under cellophane membrane.

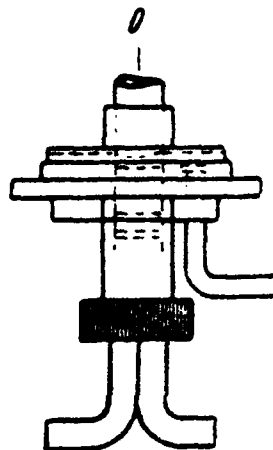


Fig. 33. General appearance of the chamber.  
O-objective.

This, in reality, applies first of all to the investigation of the presence of bacterial toxins. Along with application of the method requiring introduction of the material to experimental animals, followed by observation of clinical form of the disease developed and setting up defense experiments with specific antitoxic sera, some other methods, using no experimental animals, can be applied for the detection of bacterial toxins. Thus, Boroff and Fitzgerald (59) found out that botulinum toxin starts to fluoresce upon activation by ultraviolet rays of determined wave length. Extinction maximum is observed during radiation by waves 287 and 360 mμ long. Addition of specific antitoxic serum weakens the fluorescence of the toxin, while normal serum does not produce such effects. Consequently, a decrease in intensity of fluorescence indicates that specific toxin-antitoxin reaction is taking place.

[See next page]

Identification of micro-organisms isolated in pure cultures

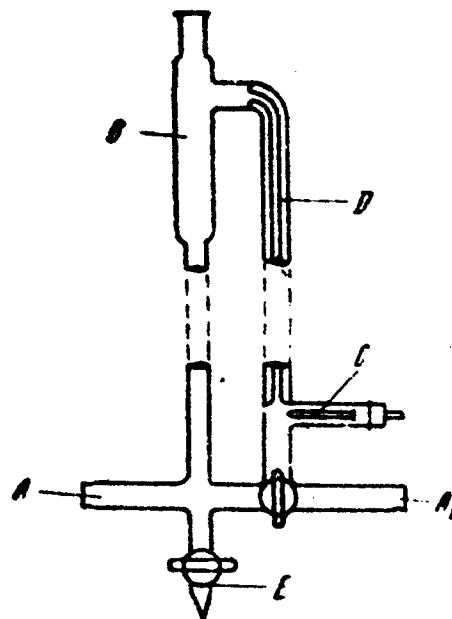


Fig. 34. Device for supplying  
the medium to the chamber  
(explanation may be found  
in the text).

can be effected by various methods - inoculation of experimental animals, investigation of morphological, biochemical and serological properties of the microorganisms, specific bacteriophage tests and many others. According to data in the cited foreign literature, the most valuable ones are those which are not only reliable, but also highly sensitive, accurate, simple and fast.

Foreign investigators working on problems of detection of biological infectious agents accord particular attention to the methods of identification of microorganisms by means of fluorescent antibodies and infrared spectrophotometry. The extent of importance attached to these methods is proven by the numerous works conducted at Camp Detrick and Porton, as well as in a number of other institutions, connected with them by a system of contracts.

Evaluating the prospects for use of these detection methods, Berger and Ludwig (60) write in a report published in the Armed Forces' Chemical Journal: "Intensive work on identification of bacteria by means of study of their assimilation of infrared radiation is now in full swing at the Robert A. Taft Sanitary Technical Center, with the collaboration of Camp Detrick personnel. There is no doubt that this method will soon become a powerful weapon of the microbiologist". They value equally highly the method based on use of fluorescent antibodies, the successful development of which, once it is concluded, will, according to them, permit identification of microorganisms within an hour and possibly even less.

The essence of this method is the fact that when an antibody united with fluorochrome is brought in contact with the preparation containing specific antigen (tissue section, smear-print, ordinary smear), it becomes attached to it, and at the point of its presence a fluorescent pigment is fixed. Observation of such preparations under luminescent microscopes allows one to determine the location of the antigen and draw a conclusion regarding its conformity with the serum used.

Methods of obtaining and applying the fluorescent sera were first developed at Coons' laboratory (61). Fluorescent immune sera are obtained by means of uniting



fluorochrome and globulin or gamma globulin fraction of the immune serum. As fluorochrome, fluorescein isocyanate, possessing a bright yellow-green fluorescence, is most frequently used.

The method of application of fluorescent sera consists of the following basic steps:

- getting ready the preparation from the material containing the agents (tissue sections are usually prepared from quick-frozen, nonfixed tissue);
- staining the preparation with a drop of conjugate placed on the tissue section, followed by washing with buffer salt solution to remove the non-fixed serum;
- study of the preparation in ultraviolet light (ultraviolet microscope, opaque illuminator);
- control investigations to determine the specificity of the reaction observed (suppression of fluorescence after preliminary treatment of the preparation with unmarked immune serum, absence of fluorescence during treatment of a smear from the same material but containing no agent, absence of fluorescence during treatment of the investigated preparation with marked non-immune or heterologous serum).

A number of authors successfully used fluorescent antibodies for the detection of virus in tissue cultures. Thus, Weller and Coons (62) detected by this method the virus of chicken pox, herpes, shingles and poliomyelitis. Buckley, Whitney and Rapp (63) obtained the same results with psittacosis virus, Buckley (64) - with polio virus; Cohen, Gordon, Rapp, Macauley, Buckley (65) - with measles virus. These authors applied the so called indirect method: they first treated the preparation under study with human serum containing specific antibodies to the agent in question, and then stained it with serum marked with fluorochrome containing the antibodies to human gamma globulin. The reason for resorting to such double layer of antibodies to the antigen is that, in case of certain viruses, it is impossible to obtain serum of sufficiently high titer by means of immunization of animals. This makes it necessary to use the serum of convalescents, whose antibody titer is sufficiently high. When specific sera with sufficiently high titers obtained through immunization of the animals are available, the "direct" method may be successfully used for purposes of detection and identification of viruses in tissue cultures. This method eliminates the necessity of preliminary treatment

with human serum, as shown by the work of Watson (66) on the example of mumps virus.

Many authors have also shown the possibility of using fluorescent antibodies for detection and identification of viruses in developing chicken embryo. Liu, Eaton and Heyl (37) were able to establish the presence of influenza virus in embryos as soon as 24 hours after their infection. Similar investigations were carried out by Liu (68) with atypical pneumonia virus.

Viruses and rickettsiae can be equally successfully detected and identified in organs and tissues of the infected experimental animals. As an example, we can present the work of Coons, Snyder, Sheever and Murray (69), who with the help of fluorescent antibodies were detecting, and determining the specificity of, rickettsia of epidemic typhus and Rocky Mountains spotted fever in exudate smears and tissue sections from the organs of infected cotton rats. Epidemic typhus rickettsia was also identified in smears prepared from internal organs of infected lice.

Fluorescent antibodies are also used for speedier identification of bacteria, by means of treatment of smears prepared from cultures or, which is even more valuable, directly from the material under investigation. Very interesting in this respect is the work of Moody, Goldman and Thomason (70). The authors treated the smears, prepared in the usual way from 24 hour old *Malleomyces pseudomallei* cultures with rabbit gamma globulin, specific for this agent, conjugated with fluorochrome. Smears of the same culture, treated with normal marked rabbit gamma globulin served as control. The experiments showed that after being treated with homologous antibodies, the *M. pseudomallei* cells start to fluoresce brightly, while the bacterial bodies in the smears stained with normal globulin have the appearance of pale shadows. Specificity of the fluorescence was checked by means of treatment of smears from 55 cultures of various bacterial strains with marked gamma globulin specific for *M. pseudomallei* and marked normal gamma globulin. All the 33 experimental strains of *M. pseudomallei*, as well as 3 strains of *M. mallei*, produced bright fluorescence after treatment with homologous antibodies, while no other bacteria produced this phenomenon. Also, in the smears treated with marked normal gamma globulin no fluorescence was observed. To prove the specificity of fluorescence, the authors tried, for 12 hours and at the temperature of 5-10°, to

exhaust the marked homologous gamma globulin with large quantities of live *M. pseudomallei* cells. Treatment of smears from culture of this agent with such exhausted gamma globulin no longer produced fluorescence. Inhibition of fluorescence also followed when *M. pseudomallei* smears were treated with unmarked gamma globulin before being stained with marked specific gamma globulin.

It was proven later (71) that owing to its high sensitivity this reaction could be used for bacterial identification not only in pure cultures, but also in mixed ones. The high degree of sensitivity of this reaction is proven by the fact that specifically stained fluorescing cells can be discovered in smears prepared from suspensions containing only 220 microorganisms in 1 ml. Experiments with mixtures of microorganisms showed that the agent of myeloidosis could be regularly detected in samples containing 200 or more cells per 1 ml, regardless of the ratio of the number of homologous cells to that of the heterologous ones. For instance, during the study of *M. pseudomallei* and *Pseudomonas aeruginosa* mixture, this ratio was 1:10,000,000 respectively.

Also successful were the experiments on detection of *M. pseudomallei* in soil samples containing a vast number of additional microorganisms. The best results were obtained from investigation of smears prepared by diluting the samples in physiologic solution, then passing them through **Watman** filters and centrifuging. In the preparations thus obtained, *M. pseudomallei* could be detected in soil samples containing as few as 20,000 cells of the microbe in 1 Gm. of the investigated soil.

Using the method worked out by the authors, it is possible to identify *M. pseudomallei* in smears taken from the surfaces infected with aerosol containing this agent. The method of experiments was very simple. Leaves treated with the aerosol were wiped with cotton moistened with 0.85% physiologic solution. The cotton was then passed over the surface of a microscope slide. The smear, dried and fixed by warming up, was stained with marked specific globulin. The experiments showed that it is possible for this method to detect *M. pseudomallei* on surfaces treated with aerosol containing only 20,000 microbes per 1 m<sup>3</sup>.

The possibility of application of fluorescent antibodies for identification of agents belonging to the *Salmonella* group is shown in the work of Thomasen, Cherry

and Moody (72).

The possibility of application of the "indirect" method of fluorescent microscopy for purposes of identification of *Br. suis*, *P. tularensis*, *Vibrio cholerae asiaticae*, *P. pestis* in smears is shown in the work of Carter and Leise (73). These authors obtained sera specific for these agents through immunization of rabbits. Anti-globulin was obtained from blood serum of a goat, immunized with normal rabbit globulin. The method of smear staining consisted of the following consecutive operations: the smear was covered with specific rabbit serum (diluted or not) for 5-10 minutes, then washed with buffered physiologic solution (pH 8) and dried in the air, after which the staining was supplemented with fluorescent rabbit globulin. 10 minutes later the smear was again washed, dried in the air and examined under a microscope in ultraviolet rays.

Comparison of the results of microorganism identification in smears from both pure cultures and their mixtures, by "direct" and "indirect" methods, showed them to be the same.

At the same time, Carter and Leise determined that upon application of high titer specific sera, cross reactions are observed (anti-*Br. suis* serum reacted easily with *P. tularensis*, the serum against *P. pestis* - with *B. pseudotuberculosis rodentii*); for this reason the authors recommend using low titer sera for the identification process, since then no cross reactions are observed.

Detection of unknown material can be carried out on one smear, treated consecutively with all the specific sera available, and adding each time fluorescent anti-globulin.

The method proposed by Carter and Leise is sufficiently sensitive: in cases when a smear contains about 25 bacteria per 1 mm<sup>2</sup>, the discovery of fluorescent cells under the microscope presents no great difficulty. Concentration of 3-5 cells per 1 mm<sup>2</sup> of the smear requires more thorough examination.

Fluorescent antibodies can find application also for speeding up the diagnosis of a disease.

Liu (74) observed that when smears prepared from the nasal mucosa discharge of **skunks** infected with influenza virus are stained with specific fluorescent

serum, some of them produce a bright yellow-green fluorescence, while other - only a greyish-bluish light (auto-fluorescence). The process of staining with fluorescent serum was slowed down if the smears had been preliminarily treated with unmarked immune serum, which points out the specificity of histochemical staining of virus stricken cells. The presence of fluorescent cells in animal nasal washings coincided with the occurrence of a febrile reaction. This method was also used for diagnosis of influenza in people (75).

During recent years the method of infrared spectrophotometry has been carefully studied with the object of using it for speeding up the microorganism identification. The possibility of application of this method is based on the fact that every organic compound has its own characteristic, individual spectrum, which can be used for its identification. All things considered, the difference among microorganisms is based on the difference in their chemical structure; this is why every genus, and in certain cases even every strain, has its own individual spectrum of absorption of infrared rays. By comparing the spectrum of a microbe to be identified with a standard spectrum, we can determine to what genus the isolated agent belongs.

The possibility of application of infrared spectrophotometry in the investigation of biological materials is shown in the works of Barer, Cole and Thompson (76), Wood (77), Blout and Mellors (78), etc.

In 1952, Stevenson and Bolduan (79) from Camp Detrick published their work, in which they show the prospects for employing this method in the field of detection. The authors conducted a study of the spectra of infrared ray absorption by intact bacterial cells, and determined that in spite of quantitative differences, caused by thickness and homogeneity of the investigated microbial smears, the microorganisms can be differentiated one from another by the character of their spectrum. They were even able to distinguish among the various strains of *P. tularensis*.

Stevenson and Bolduan (79) came to the conclusion that "the use of infrared absorption spectra is very promising for purposes of microorganism identification. A necessary condition for such identification is the composition of a catalog of spectra of microorganisms".

British investigators in Porton (80) later arrived at the same conclusion. They showed sharp specificity of the absorption spectra in various organisms. It was found possible to differentiate between the strains of *B. coli*, *B. pestis* and even strains of *Salm. typhi*, which were very difficult to tell apart by the methods of classic bacteriology. The method of investigation recommended by the authors was not very complex. The microorganisms to be investigated were grown on peptone agar for 18-24 hours, after which the culture was washed off. This, according to the authors, must be done very carefully, in order not to wash off, along with the culture, pieces of the medium. The microbial suspension should be removed from agar as early as possible, in order to avoid passing the soluble components of the medium into it. Inactivation of the microbial suspension was achieved by heating or autoclaving (for sporulating forms). This stage is necessary as a safety precaution for the investigator in carrying out the subsequent manipulations. The cultures can also be grown on liquid nutrient media, with subsequent sedimentation and washing off the microbial cells by centrifugation. The smears are prepared on polished silver chloride slides; glass and quartz cannot be used because of their high degree of absorption of infrared rays.

The microbes are spectrophotometrically examined in a dry state, which is also connected with the water absorption of infrared rays. The authors stress that the microbial suspension should be dried very carefully, since too prolonged drying is followed by a change in spectrum.

Checking of the constancy of spectra obtained for the same strain has shown that their reproduction depends largely on the culture medium. This is particularly illustrated by the diversity of spectra depending on whether the microorganism has been cultured on peptone agar or on peptone water. The length of cultivation also has a definite bearing, which is especially clearly evident in some strains.

The spectrogram changes, depending on the composition of the medium and the cultivation time, are fully understandable and occur in connection with the diversity in chemical structure of the microorganisms, caused by difference in the culturing process.

One of the factors influencing the reproducibility of the spectrum is the accuracy of the instrument (infrared

spectrophotometer) used for taking down the characteristics of the microorganism under investigation.

Infrared spectrophotometry can be used not only for study of whole cells, but also for that of various extracts prepared from them. Stevenson and Levin (81, 82, 83) developed the method of extraction and carried out the first spectrophotometric studies of microbial extracts. Schneider and Laughlin (84) showed the possibility of differentiation of Leptospirae into serogroups on the basis of absorption spectrum differences among the extracts prepared from them. Randall and Smith (85) measured the absorption spectra of methyl chloroform lipid extracts of Mycobacterium tuberculosis cells and discovered spectroscopic discrepancy between the hominis and bovis types.

Detailed investigation regarding the possibility of applying the method of infrared spectrophotometry of microbial extracts and development of a simple and standard method for agent identification were carried out by O'Connor, McCall and Dupre (86) of the New Orleans Scientific Investigation Laboratory of the southern region, under contract from Camp Detrick.

The method developed by these authors consisted of several steps:

- 1) cultivation on agar media for 18 hours;
- 2) disintegration of cells by means of treatment in a special homogenizer with finely ground glass;
- 3) acetone extraction of the disintegrated cell mass at 50°-60° for one hour, with subsequent centrifuging and evaporation of the centrifugate over a water bath. The evaporation is continued until only a few drops of the centrifugate remain;
- 4) preparation of smears on silver chloride plates and drying them by means of infrared radiation;
- 5) measuring infrared absorption spectra in the spectrophotometer, at various wave lengths (5-15  $\mu$ ). The necessity to use various wave lengths is dictated by the fact that, as pointed out by Levine, Stevenson, Chambers and Kenner (87), the absorption zones in the range of 6.5-6.45  $\mu$  are mainly associated with peptide chains of microbial protein, the absorption zones in the range of 8-8.1  $\mu$  - with nucleic acids and the absorption zones in the range of 8.6-10  $\mu$  with carbohydrates and nucleic acids. Thus, the use of different wave lengths permits a fuller understanding of the microbial structure and shows the differences among microorganisms more clearly.

6) comparing spectra of the extracts of the investigated microorganisms with those that are already known.

Comparing the method of investigating the extracts with the method of spectrophotometry of intact bacterial cells, the authors came to the conclusion that the former is more promising, for it makes it possible to differentiate between microorganisms possessing very close or even identical infrared absorption spectra, obtained by spectrophotometry of the intact cells.

However, it must be noted that for certain bacteria acetone is inconvenient as an extracting substance, and has to be replaced by some other solvent. If acetone extracts one or more compounds which are not common to the microorganisms to be differentiated, or if it extracts the substance common to these two microorganisms, but present in one of them in a higher concentration, then it is relatively easy to tell these microorganisms apart by their spectra. However, in the case when acetone extracts only substances which are present in both microorganisms under study in equal proportion, the infrared absorption spectra will be identical. This circumstance made it necessary to abandon the extract spectroscopy method, since the necessity of solvent selection greatly complicates the process of analysis and requires a great number of microbial cells, the recovery of which will considerably protract the time of analysis.

The infrared spectrophotometry method can, evidently, be used also for identification of microbial toxins, the principal possibility of which is shown by the investigations of Levi, Matheson and Thatcher (88). The virus problem is more complicated. Pollard, Engley, Redmond, Chinn and Mitchell (89) studied the infrared absorption spectra of meningopneumonitis, ornithosis, mumps and Newcastle virus. Chorionallantoic fluid of infected chick embryos served as material for investigation. Comparison of the spectra showed that mumps and Newcastle virus are easy to differentiate both from one another and from meningopneumonitis and ornithosis virus. The last two, however, have very similar spectra, which greatly complicates their differentiation. This is explained by antigen affinity of both of these agents.

Further investigations of this problem uncovered many more difficulties in virus identification with the help of infrared spectrophotometry. Benedict, Pollard and



Engley (90) tried to use this method for differentiation among the viruses of influenza A and B, mumps, meningo-pneumonitis, Newcastle disease and psittacosis. These experiments, like the previous ones, were carried out on chorionallantoic fluid of infected chick embryos, which was previously subjected to three cycles of differential centrifugation. On the basis of these investigations, the authors came to the conclusion that the use of untreated fractions for virus identification by the method of comparing infrared absorption spectra is greatly complicated by the presence of extraneous material in the investigated substrate; however, the three cycle treatment permitted to obtain clear and reproducible results for the meningo-pneumonitis and Newcastle virus, but the spectra of influenza A and B and parotitis virus as well as of normal chorionallantoic fluid were found to be identical even after the differential centrifugation.

Afterwards Benedict (9) showed that for differentiation of related viruses it is necessary to combine centrifugation with extraction, for which the solvent has to be individually selected in each case. However, the 3-cycle centrifugation is sufficient to determine to which virus group the agent in question belongs. The viruses studied by the author - influenza strains A, A<sub>1</sub> and B, Newcastle disease, parotitis, psittacosis, lymphogranuloma venereum, feline pneumonitis, mouse pneumonitis, vaccinia and fowl-pox - fall into 4 groups, according to the infrared absorption of the preparations obtained from them: group I - viruses of influenza A, A<sub>1</sub> and B and parotitis; group II - Newcastle disease virus; group III - psittacosis, lymphogranuloma venereum, feline pneumonitis, mouse pneumonitis; group IV - vaccinia and fowl-pox.

A study of the problem of using infrared spectrophotometry for identification of microorganisms was taken up by Riddle, Kabler, Kenner, Bordner, Rochwood and Stevenson (92) on contract with Camp Detrick. Their object was to develop a method of producing infrared absorption spectra (of the microorganisms under study), possessing a very high order of quantitative reproducibility, and to study the possibility of coding standard spectra on punched cards for subsequent processing in electromechanical and electronic equipment.

Through standardization of the bacterial culturing conditions, exact spectrophotometer adjusting and using the method of preparation of films of necessary thickness from bacterial suspensions, the authors were able to eliminate

the discrepancies from spectra of one and the same culture. The differences between the reproduced spectra did not exceed  $\pm 0.2\%$ .

As the result of repeated adjustments of different parts of the spectrum, the discrepancies among the spectra of various strains of the same microbial species were reduced to a minimum.

The most favorable wave range (7-12.1  $\mu$ ), in which the best reproduction could be achieved, was also determined.

The results of investigation of a great number of different microbial species showed that the spectra of many of them differ considerably and that this fact can be used for identification purposes. Some microorganisms, however, have such similar spectra, that their differentiation cannot be achieved with the help of only spectrophotometric analysis.

The code system developed by the above authors makes it possible to record rapidly and easily a bacterial spectrum on cards, together with morphological and tinctorial properties, culture conditions and many other data. The use of such cards allows the use of electro-mechanical and electronic equipment, with the help of which a great quantity of standard spectra and other differential data related to them can be sorted out, investigated and compared.

The code-cards developed during the course of the work and accepted as standard, formed the nucleus of the future catalog of standard spectra.

The authors visualize the following order of identification of microbes with the help of infrared spectrophotometry. The microbe to be identified is inoculated on several plates with various media used for cultures of bacteria, the standard spectra of which are in the catalog. The microorganisms for investigation are gathered from the plate which has produced the most abundant growth. At the same time a Gram stained smear is prepared. For comparison with the investigated microbe only these cards are removed from the catalog, which contain spectra of microbes having the same stain and morphology and grown on the same medium. The cards are placed in a sorting machine, which effects the comparison of spectra. Electronic equipment gives the best sorting performance.

According to the calculations of Williams and Ingraham (93), the whole process of detection, starting from the moment of sample collection, until the species of the microorganisms present in it are identified, will take not more than 30 hours with the use of infrared spectrophotometry. Membrane filters, on which the early growth takes place for 10-20 hours, are recommended by the authors for use during the course of the investigation. Suspicious colonies are transferred to a plate with standard medium. The indispensable time of its incubation does not exceed 6-8 hours, since a very minute quantity of bacteria is sufficient for spectrophotometric analysis (1 mg., and possibly 0.1 or even 0.01 mg., as shown by the most recent investigations).

Evaluating the performance of the biological infectious agent detection methods developed to-date, American specialists arrived at the conclusion that, in spite of the known achievements in this field, it will not nearly always be possible to count on a timely answer to the question of whether a bacteriological weapon has been employed and which agent has been used by the enemy. While there already exist methods which, if correctly applied, will permit the timely (i.e. before the appearance of the disease) detection in the objects of external surroundings of a number of bacterial agents such as *P. pestis*, *P. tularensis*, *Br. melitensis*, there is no such possibility for viruses and rickettsiae. Consequently, diseases caused by the application of a bacteriological weapon can appear before the agent is identified (94).

Certain biological infectious agents cause diseases with a very short incubation period, which makes it difficult to rely on the possibility to effect detection in such a short time. Thus, upon application of the virus of Venezuelan equine encephalomyelitis, some cases appear within 24 hours; upon employment of botulism toxin - within 8-12 hours. Consequently, in certain cases the appearance of disease will be the first sign of the employment of a bacteriological weapon. It is for this reason that the special American manual "Civil Defense against Biological Warfare" (1) points out simply: "Until the moment of development of reliable physical or biological methods for determination of contamination of the environment, man will remain the most dependable detector of the biological agents directed against him".

### Organization of the detection system in the U.S.A.

Present organization of the system of detection of biological agents in the U.S.A. has the following structure (95):

The most important districts of the country are under continuous observation by the Air Force Anti-Aircraft Defense and Civil Defense Observation Posts. Civil Defense Voluntary Ground Observation Corps was created in 1950. In 1952 it counted 145,000 men and supplied day and night service at 14,000 observation points. According to specialists, in order to carry out a successful observation service, manpower of the Ground Observation Corps should reach the 500,000 figure. The Observation Posts are equipped with simpler of the instruments for air sample collection, which they use whenever a suspicion of bacteriologic attack arises.

Bacteriologic exploration is also carried out by State Health Department's Mobile Laboratories and by Special Flying Units, which belong to the Civil Defense District Diagnostic Laboratories. The function of Mobile Laboratories Flying Units is the collection of samples and effectuation of simpler analyses. They consist of one physician-bacteriologist and three laboratory technicians. The task of sample collection also rests on the local commands of bacteriologic exploration, organized from volunteers in each community.

The investigation of samples collected is carried out at State Central Laboratory, which is connected with the Civil Defense, at local Health Department bacteriologic laboratories in the cities, and in the laboratories of hospitals, clinics and other medical institutions. When necessary, laboratories of the Center for Hygiene and Infectious Diseases, National Institute of Microbiology and other scientific research institutions and universities are also expected to take part in the work. In addition, each state plans the creation of a specialized laboratory for the detection of rare and insufficiently known infections.

It is believed that Civil Defense District Laboratories, as well as State Health Department's local laboratories will provide only a preliminary answer about an analysis. More reliable laboratories, which possess the special equipment and staffs of qualified bacteriologists, will be called upon for final identification of the agent.

The latter institutions include State Central Diagnostic Laboratories, laboratories of the Center for Hygiene and Infectious Diseases and the National Institute of Microbiology, as well as the laboratories of other scientific research institutes, universities and clinics.

Since the appearance of disease is considered as one of the first signs of employment of a bacteriologic weapon, a great attention is paid in the U.S.A. to the diagnosis and registration of all appearing cases of infection. A whole series of articles devoted to this problem, as well as special manuals and instructions stress that not only employees of the Health Service organs, but also administrative workers, teachers and other persons, whose nature of employment brings them in contact with organized collective groups, should take active part in reporting the sick.

Because the discovery and diagnosis of infectious diseases represent one of the more important methods of detection of biological infectious agents in the bacteriological defense system, the method of information about the movement of infectious morbidity in the U.S.A. acquires special importance. Federal Civil Defense Administration and a number of Government Departments (Health, Agriculture, Social Security), together with the National Bureau of Birth and Death Statistics, came to an agreement regarding the creation of a system of organs, the duty of which would be to observe the appearance and movement of human, animal and plant infectious diseases. At the Central Anti-epidemic Administration of the Department of Health, a special anti-epidemic service has been created, its duties including the publication of data on infectious diseases, familiarization of interested persons with the problems involved and popularization of the information about anti-epidemic measures to be taken.

The organization of a system for detection and identification of the biological agents used requires the training of qualified bacteriologists and epidemiologists possessing up-to-date information and know-how. Consequently, in 1950 the Federal Civil Defense Administration undertook the training of numerous laboratory personnel in the methods of detection.

The preparation of epidemiologists has been entrusted to the Anti-epidemic Information Service of the Central Anti-epidemic Administration, which started this work in 1951. The epidemiologists attended a comprehensive

course in epidemiology, biostatistics and health organization, followed by field practice. The persons who finished this preparatory course could continue working as epidemiologists or be employed in any other capacity. It must be assumed that a part of these specialists returned to their particular jobs or to work at scientific institutions or clinics.

It is understood, however, that at the time of war they will be called up again as Health Service epidemiologists and directed to work in the districts of strategic importance.

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## INDIVIDUAL PROTECTION AGAINST THE BACTERIOLOGICAL WEAPON

According to American direction and supervision (1,2), means of specific prophylaxis and therapy of infectious diseases (vaccines, therapeutic sera, antibiotics) as well as nonspecific means of protection (gas mask and protective clothes) pertain to individual means of protection of the population against bacteriological warfare.

### Specific means of protection

#### Vaccines

Vaccines are considered in the USA as a powerful means of antibacterial protection, which when applied in time, along with immunization, in case of epidemic indications, can, to a considerable extent, prevent, limit the distribution and alleviate the course of infectious diseases, arising as a result of a bacteriological attack. Therefore, the problem of vaccine prophylaxis is the center of attention and is studied earnestly along the line of working out a rational plan of immunization of the armed forces and of the population with the purpose of establishing in them a "basic immunity", as well as for discovering new vaccines and improving existing vaccine preparations against infections, the causative agents of which might be applied as bacteriologic weapons. (3,4)

However, as admitted by American specialists, the USA at the present time does not have at its disposal vaccines against all the diseases, the causative agents of which might be used in bacteriological warfare (5,6). Moreover, the opinion is expressed that many of the existing vaccines will not be able to secure protection of the population in case of massive doses of the infective agent and of unusual ways of infection, i.e., under conditions which can take place in times of bacteriologic warfare. Finally, the absence of associated vaccine preparations, the creation of which was started relatively recently in the USA (4,6), and the lack of qualified personnel for carrying out vaccinations can significantly impede the execution of mass-immunization in short periods of time. The circumstances, mentioned above, as well as fear of causing discontent among the population do not permit, at the present time, carrying out, in the USA, the program of mass-immunization of the population and armed forces against infections, which is urgent from the standpoint of bacteriologic warfare. Therefore, in the USA at the present time, the attention of public health agencies is called only to the problem of increasing the work of immunization of the population against "natural"

infectious morbidity (natural smallpox, tetanus, typhoid fever and paratyphoid fever, diphtheria, whooping cough), which will make it possible to produce an immune stratum and secure a decrease of morbidity of these forms during the war, when the general material and sanitary-hygienic level of the population will be significantly decreased. However, it is supposed that, side by side with these measures, under conditions of war, by establishing the species of the applied causative agent, the population of the affected area, or even of the whole country, will be subjected to active immunization against the infection, the causative agent of which has been used by the enemy. The offices of Civil Defense are made responsible for carrying out these measures (7).

American research institutions, simultaneously, work intensively on improving the existing preparations and on developing new highly effective ones, against all infections, the causative agent of which might be used as a bacteriological weapon; wide research is being carried out for creation of chemical and associated vaccines (4).

In the present chapter, the system adopted in the USA and many other capitalistic countries, of active immunization of the armed forces and of the population, is described briefly, the characteristics of vaccine preparations used there are presented, and directions are also elucidated, in which the improvement of old vaccines and the creation of new ones representing interest from the standpoint of antibacterial protection is carried out.

In the USA as well as in most other countries, protective vaccinations can be divided into planned vaccinations, carried out systematically, and vaccinations organized on epidemic indications.

In the armed forces of the USA, the planned vaccinations are carried out against natural small-pox, typhoid and paratyphoid fever A and B, tetanus, and diphtheria. In case of epidemic indications, vaccination is provided against yellow fever, exanthematous fever, cholera, plague and influenza, Rocky Mountain spotted fever, Q-fever, and a series of less frequent infections: psittacosis, equine encephalomyelitis, Japanese encephalomyelitis, etc. (1,2,4).

There is no law for compulsory, planned vaccinations of the civil population in the USA at the present time, and vaccine-prophylaxis is carried out by the population at their own wish, according to the recommendations of the public health agencies.

As planned vaccinations for the population are recommended:

- vaccinations against natural small-pox for the whole population, especially prior to a trip abroad;

- vaccinations against typhoid fever and paratyphoid fever A and B for persons living in unsanitary conditions and in endemic foci;

- vaccinations against tetanus for the whole population, starting with childhood;

- vaccinations against diphtheria for the whole population, starting with childhood and continuing to the age of 35, until the Schick reaction becomes negative; this vaccination especially is recommended in case of trips to Europe and to the Mediterranean coast;

- vaccinations against whooping cough for children up to the age of 5;

- vaccinations for children against poliomyelitis.

In case of epidemic indications the population of the USA is immunized against the same infections as the armed forces (8).

Recently the public health agencies in the USA have widely discussed the question on the introduction of compulsory vaccinations against certain infections. In many states (Arkansas, Maine, New Mexico, West Virginia) laws have been adopted for compulsory vaccination of children against small-pox and also for immunizing them against diphtheria (Maine, West Virginia) (9).

The system of planned vaccination, and also vaccination in case of epidemic indications, adopted in French Army and Navy, do not differ from those in the USA.

The whole civil population in France, starting from childhood, is subjected to compulsory and planned immunization (legalized by corresponding governmental resolutions) against the same infections as the population in the USA. In case of epidemic indications, vaccinations are carried out against the same infections as in the army.

Further in this presentation the characteristics of vaccine preparations used for planned immunization and also of vaccines having essential significance as means of specific protection against bacteriologic weapons are given. In view of the fact that all measures for antibacterial protection, including immunoprophylaxis, are constructed on the basis of systems of anti-epidemic protection existing in peace time, the presentation of the material is written up, not in order of the importance of the infection as a bacteriologic weapon, but beginning with the characteristics of the vaccine preparations used in the planned immunization.

As it follows from the above statements, vaccines against natural small-pox, typhoid fever and paratyphoid fever A and E, tetanus and diphtheria are used for planned vaccinations in the USA.

Natural small-pox. The causative agent of natural small-pox is acknowledged as one of most probable agents in the bacteriologic war. Since effective therapeutical means for this disease have not been worked out up to the present time, and the lethality and contagiousness of natural small-pox is very high, vaccine-prophylaxis of small-pox takes the leading place in the combat with this disease (see chapter IV).

The high efficiency of active immunization against natural small-pox according to the method of Jenner is well known (8,10). Thanks to its application, significantly more human lives were saved and more illness was prevented than from carrying out all remaining vaccinations taking together. Systematic vaccination against small-pox has secured the liquidation of small-pox in most countries of the world and has also brought about conditions for extinction of epidemics and pandemics of this disease.

Vaccination gave good results during the war in Korea, where small-pox appeared to be endemic. Preliminary data on the American army, the personnel of which was vaccinated, show that out of every 100,000 men in the infantry, there were 4 cases of the disease; in the marines, 7 cases were registered, and in the navy and military air force, one man got sick. Among the war prisoners, (also vaccinated) out of every 100,000 men, 5 were sick. At the same time, among the Korean civilians, not vaccinated against small-pox, epidemics took place; in the winter of 1952, especially, an epidemic among children was observed, including 300 persons (8).

Observations in regard to natural small-pox during recent years prove convincingly the high efficiency of vaccinations, and also the necessity for carrying out routine specific revaccinations. Underestimation of this requirement creates conditions for the appearance and spread of the disease. Thus, in 1947 in New York, where, as in the whole country, vaccination is not required by law, an outbreak of small-pox developed including 8 people (11). One of the reasons for its appearance seemed to be the irregularity of vaccination and revaccination, which caused a decrease of the level of vaccination immunity in the population of the city. In a short period of time, about 5 million people were subjected to vaccination. Mass immunoprophylaxis, carried out together with other anti-epidemic measures, made it possible to check further spread of the disease and to liquidate the outbreak.

In 1954-1955 in France (Bretagne) an epidemic, including approximately 200 people appeared, simultaneously, individual cases of the disease were recorded in the American forces in France (12,13,14).

It was established that the disease appeared and took the most severe course in persons not vaccinated against small-pox (evaded compulsory vaccination) or who were vaccinated more than 10 years ago.

Small-pox vaccine, produced in the USA is standardized by the National Institute of Hygiene and represents a suspension of the cow's small-pox virus in glycerin, grown on scarified calf's skin. The preparation is released in a liquid form and is kept without losing activity at a temperature below 0° for 3 months. Vaccination is performed by the method of scarification (1 drop). The duration of the immunity is 4-5 years; revaccination is carried out after every 4 years and, in addition to this, in case of epidemic indications. The reaction to small-pox vaccine is usually insignificant, however, during mass-immunization of the population of New York in 1947 (5 million people) in 45 persons the phenomenon of post-vaccine encephalitis was observed (15,16).

Within the past few years in the USA, a dry small-pox vaccine has been experimentally worked out and tested on humans; it was prepared from the membrane of chick embryos infected with standard small-pox virus from calf's lymph. The preparation has a high effectivity and may be kept in an active condition for a long time. Steps are taken at the present time for introducing this new preparation into anti-epidemic practice (17,18,19).

In England, small-pox vaccine is released in a dry form, with storage time up to 12 months (storage in dark, at temperature below 0°) (20).

In Switzerland three types of vaccines against natural small-pox are prepared: 1) lymphovaccine, treated with glycerin (analogous to the vaccine released in the USA); 2) lymphovaccine in chemically pure lanolin, resistant to high temperature, applied in the tropics; 3) dry lymphovaccine, applied in the tropics; this can be stored even better at a high temperature (21).

Vaccines against small-pox, in general, are made in most of the capitalistic countries and are used for planned immunization of the population and armed forces. In certain countries associated preparations have been obtained against small-pox and typhoid fever (Japan), small-pox and yellow fever (USA) (22,23,24). The vaccination secures the development of intense immunization, which successfully protects from disease in case of natural infection.

The question of the ability of the small-pox vaccine to protect the human body, contaminated by means of aerogenic infection with a massive dose of virus, is open.

Typhoid fever and paratyphoid fever A and B. The causative agents of typhoid fever and paratyphoid fever A and B as agents of bacteriologic war are of slight significance in the opinion of American investigators: therefore planned immunization of armed forces and of the population against these infections is carried out only to combat natural morbidity (see chapter IV).

In the USA army compulsory vaccination against typhoid fever and paratyphoid fever A and B was introduced since 1911 (2). Immunization among the population is recommended for persons living and temporarily staying in endemic areas, as well as for persons in danger of infection because of their profession (8). Also, persons being in contact with diseased persons are vaccinated.

An associated vaccine representing a suspension of killed acetone-dehydrated microbes is adopted as a vaccine preparation against typhoid and paratyphoid fever A and B for supplying public health agencies and the armed forces in the USA (15,16,25). For the preparation of the vaccines the Panama strain 58, and the English strain Ty<sub>2</sub> are used. The vaccines are supplied in ampules of 50 ml.; 1 ml. contains 1 billion typhoid and 250 million paratyphoid microbes A and B. By using acetone for the treatment of a microbial mass with subsequent lyophilization, American authors have accomplished the preservation of Vi-antigen (as well as of H- and O-antigens) important from the immunologic point of view.

The course of immunization, adopted in the USA, provides for three subcutaneous injections of the vaccine of 0.5 ml. each, at intervals of 7 to 28 days (8). Tufts recommends an "alternative method": three intradermal injections of 0.1, 0.2 and 0.2 ml. each. Among the civilian population, a revaccination according to this scheme is carried out after 4 years, but in the army - after 3 years; for persons living in areas endemic with regard to typhoid fever, as well as for medical personnel and workers in food industries and laundries, annual revaccination of 0.5 ml. subcutaneously, or of 0.1 ml. intradermally is recommended. The vaccination is accompanied by general and local reactions; however, they take a light course. In intradermal immunization, reaction to the vaccine is insignificant. The duration of the immunity produced is not determined exactly; however, it lasts not less than a year.

The introduction of compulsory immunization against typhoid and paratyphoid fever A and B in the armed forces of the USA was accompanied

by a sharp decrease in the morbidity from these infections. Thus, already during World War I, the average morbidity from typhoid fever in the American army was 0.37 cases per 1000 people, while in the French army, where immunization had not yet been introduced, the morbidity reached 14.86 per 1000 people (26)<sup>1</sup>. During World War II, only 677 cases of typhoid fever were registered in the American army.

Experiences with immunization of the armed forces of the USA against typhoid fever and paratyphoid fever, during the war in Korea are indicative of the relatively high efficiency of the vaccine preparations applied. According to the preliminary data, out of every 100,000 men in the land forces, only one case of the disease was registered, and out of every 100,000 marines - 2 cases; no morbidity was registered in the military air force nor in the navy (8).

However, in the opinion of many American authors, the improvement of the typhoid fever vaccine (treatment of microbes with acetone, securing the preservation of Vi- antigen), nevertheless did not justify the hopes set upon this. The decrease in the annual morbidity from typhoid fever in the country to 2250 cases they are inclined to be connected rather with the improvement of sanitary-hygienic conditions than with the high efficiency of vaccination. In order to confirm this point of view, we can mention Miller's data on two outbreaks of typhoid fever in Suez among English pilots in whom, in spite of general vaccination with a vaccine prepared according to the method adopted in the USA, a morbidity of 30-40% of the army personnel was registered, whereupon the lethality constituted 10-11% (27).

Further improvement of immunoprophylaxis against typhoid and paratyphoid fever A and B is carried out with the use of purified Vi- and O- antigens as typhoid fever vaccines. Working with these antigens, isolated from *S. typhosa* 0-901 and *Escherichia coli* 5396/35 respectively, Landy (1949) established that a single subcutaneous introduction of 20 ugm. O-antigen and 40 ugm. Vi-antigen secures the accumulation of a higher level of antibodies in the blood of the immunized person than a full course of vaccination (three injections) with a heated or acetone-dehydrated vaccine. Another advantage of the new preparation is its lower tendency to cause reactions (28,29). According to the report by Edsall (3), the American vaccine of purified Vi- and O-antigens was tested in an epidemiologic trial in 1954 and 1955 in Yugoslavia, whereupon the preliminary results were found quite satisfactory (4).

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<sup>1</sup> In comparing these figures, it is also necessary to consider that, during the war, the French army was under more difficult epidemiologic conditions.



In England, for prophylaxis against typhoid and paratyphoid fever, trivaccine TAB, prepared from microbes (strain Ty<sub>2</sub>) which are killed by heating as well as by treatment with ethanol, is used at the present time. The treatment with alcohol, according to English authors, makes it possible to better preserve the antigenic properties of the microbes. The vaccine contains 1 billion typhoid and 500-750 million paratyphoid microbes A and B per 1 ml. The preparation is applied subcutaneously twice in doses of 0.25 and 0.5 ml. (alcohol vaccine) with an interval of 7-28 days. The vaccine killed by heating, is introduced in the same way, but in doses of 0.5 and 1 ml. A booster shot is recommended after 6 months. Immunity is maintained for 1-3 years. The injections are accompanied by a moderate local and systemic reaction. Prophylactically, the English vaccine corresponds approximately to the American vaccine. The vaccination lowers the morbidity from typhoid fever and paratyphoid fever A and B, but does not guarantee against the possibility of disease, which, however, takes a lighter course than usual (20).

In France, along with other associated preparations a vaccine against typhoid fever and paratyphoid fever A and B is supplied; it is prepared from microbes killed by heating. One ml. of the preparation contains 750 million typhoid, 250 million paratyphoid A, and 500 million paratyphoid B microbes. Immunization is carried out with three subcutaneous injections of 0.5, 1 and 1 ml. at intervals of 7-10 days. Revaccination is carried out after 2 years with a dose of 1.5 ml. Further, the revaccination should be repeated every 4-5 years. The vaccine gives a low local and systemic reaction.

Vaccinations against typhoid fever with a monovaccine has been carried out in France since World War I; since 1931 compulsory immunization of the armed forces with an associated vaccine against typhoid fever, paratyphoid fever and diphtheria was introduced; since 1936 vaccination against tetanus has been added to the immunization system - the tetanus component is introduced into the vaccine. During World War II in Algiers an associated vaccine against typhoid fever, paratyphoid fever A and B, diphtheria, tetanus and exanthematous fever was prepared (30).

In conclusion, it is necessary to mention that a vaccine against typhoid fever and paratyphoid fever A and B is produced in most foreign countries and is supplied either in the form of trivaccine (TAB), or in the form of more complex preparations. An ordinance for immunization against typhoid-paratyphoid diseases at the present time exists in most foreign armies and is carried out, in addition to the above mentioned countries, in Italy (starting with 1939), in Canada (since World War II), Brazil, Portugal, Turkey and other countries. In Japan for immunization of "safety forces", starting with 1949, a polyvalent vaccine against typhoid-paratyphoid fevers and dysentery has been

applied; even earlier, the application of an experimental vaccine was begun against typhoid fever and natural small-pox.

Summary data, characterizing the epidemiologic efficiency of vaccines against typhoid fever, are presented by Edsall in a form of table (4) (table 6).

Table 6

Year	Number vaccinated	Number non-vaccinated	Morbidity indications (in %)	
			Among the vaccinated	Among the non-vaccinated
1913	10,378	8,936	0.54	3.2
1915	--	--	0.095	1.03
1943	4,000	800	1.4	7.0
1944	170	531	4.7	11.6
1946	180	194	0.5	8.7

**Tetanus.** Although the probability of application of tetanus toxin as a bacteriologic weapon, in the opinion of American specialists, is quite doubtful, a planned immunoprophylaxis against this disease has great defensive significance, since tetanus appears as a typical wound infection in war time (see chapter IV).

Tetanus anatoxin appears to be one of the most effective prophylactic preparations and is successfully used for routine vaccination in most foreign armies. In the USA and England, vaccinations are carried out with tetanus anatoxin. In France, Italy, Canada, Switzerland, Portugal, Brazil and Turkey, tetanus anatoxin is included in associated vaccines, containing, besides, components against typhoid-paratyphoid fevers.

Experience during World War II showed convincingly that the application of tetanus anatoxin in the armed forces in many belligerent countries secured a significant decrease in morbidity from tetanus in comparison with World War I, when the wounded, at best received serum only for prophylactic purposes. Thus, for instance, in the English army, the mortality from tetanus decreased from 1.5 to 0.06 of 1000 wounded. In the American army, during World War II, only 12 cases of disease were registered out of 10 million mobilized men, whereupon 6 of these were not vaccinated. It should be emphasized especially that serum prophylaxis was not carried out on the wounded, subjected to previous vaccination with anatoxin. In the Australian army, only 13 cases of tetanus were registered, and only one case ended fatally (the army - 587,000 people). In the Canadian army, 3 cases of disease were registered with one fatal outcome. In the French army, during the campaign of 1939-1940, as well as in the Brazilian corps during the

campaign in Italy, not a single case of this disease was registered.

At the same time in the German army, where vaccination against tetanus was not carried out, and only serum was injected into the wounded for prophylaxis, in the operations in Normandy alone (1944), 80 cases of tetanus were registered. In Manila, during the war operations, 400 cases of tetanus among the nonvaccinated civilians were registered.

Tetanus was observed in the Japanese army, where vaccination was not carried out.

In connection with the high prophylactic efficiency of tetanus anatoxin in many countries after WW II (USA, France) laws of compulsory vaccination of the civilian population, starting with childhood, have been introduced (31,32).

In the USA, for immunization against tetanus, a tetanus anatoxin precipitated with alum, as well as a native tetanus anatoxin is used. The precipitated anatoxin is introduced intramuscularly in the amount of 0.5 ml. twice with an interval of 1 month (better after 2-3 months); the native anatoxin is applied three times, with the same interval in a dose of 0.5 ml. subcutaneously or intramuscularly. Revaccination is carried out after a year with a dose of 0.5 ml.; afterwards, vaccinations are repeated after every 4 years (each time 0.3 ml. of the deposited preparation is used intramuscularly or 0.1 ml. intradermally). Revaccination is also indicated in case of large and torn wounds, animal bites, burns and other contaminated injuries, as well as in any work where danger of contamination with tetanus spores exists.

Persons, who have not had primary vaccination and are in need of extra serum prophylaxis, are simultaneously subjected both to active and to passive immunization (in different parts of the body!)

Serum prophylaxis against tetanus in vaccinated persons is recommended only in cases of large wounds or when first aid was rendered too late (after 24 hours or later); in such cases, simultaneously with anatoxin, 10,000 AU of serum are introduced. If, however, prophylaxis can be initiated immediately, it is considered to be sufficient to introduce a revaccination dose of anatoxin.

At the present time, work is being carried out in the USA for the creation of complex preparations against tetanus, diphtheria and whooping cough (8,15,16,33,34,35).

Diphtheria. The causative agent of diphtheria and its toxin, just as the causative agents of typhoid and paratyphoid fever, and tetanus, does not belong to the probable agents of bacteriologic warfare. There-

fore, immunization against diphtheria pursues only the object of combating "natural" morbidity (see chapter IV).

In the USA, routine immunization against diphtheria is compulsory in the armed forces and is recommended for the whole civilian population, starting with childhood and continuing up to the age of 35, in case of a positive Schick reaction. Immunization is carried out with deposited diphtheria anatoxin (adsorbed on aluminum hydroxide, precipitated with aluminum phosphate or with alum) after a preliminary application of Schick's test. If the reaction is weak, anatoxin is introduced subcutaneously in doses of 0.1, 0.5, 1 and 1 ml.; in case of moderate reactions, 4 injections are carried out: 0.1, 0.3, 0.5 and 1 ml.; the second dose is introduced 48 hours after the first one, but the following doses - after 3-4 week intervals. A very definite Schick's reaction is a counterindication for vaccination. The duration of the preservation of immunity is not definite; however, it is measured in terms of several years. Revaccination is recommended to be carried out every 4 years by means of subcutaneous injection of 0.1 ml. anatoxin; it is also recommended to apply some associated preparations consisting of 2-3 components (tetanus and diphtheria; tetanus, whooping cough and diphtheria) which secure an increase of the immunizing properties of diphtheria anatoxin (1,2,8,25).

Such, briefly, are the characteristics of bacterial preparations which are used in the USA, and certain other countries, for carrying out routine immunization of the armed forces and of the population. As was indicated, vaccinations against other infectious diseases are carried out in case of epidemic indications. Below are listed data on immunoprophylaxis of only those infections, the causative agents of which, according to data of American authors, can be used as bacteriologic weapons.

Plague. According to the unanimous opinion of American specialists, the causative agent of plague appears to be one of the most probable, and, very likely, most dangerous agents in bacteriological war (see above). In view of the extreme contagiousness and high fatality of plague (especially in the pulmonary and septic forms) even if the most modern methods of specific therapy are applied, vaccine-prophylaxis of this disease under conditions of bacteriologic war acquires primary significance. Naturally, the preparation selected for this purpose must be highly effective; in particular, the problem is raised concerning its ability to protect against aerogenic infection with massive doses of virulent cultures of plague.

Vaccinations against plague in the USA are adopted for the armed forces as well as for the civilian public health system (1,2,8). Immunization is indicated, above all, in cases of trips to areas of endemic (enzootic) plague or upon the appearance of epidemics or

enzootics. It is assumed that vaccination against plague will also be carried out under conditions of bacteriological warfare by using the causative agent of this disease. For active, specific prophylaxis in the armed forces in the USA a vaccine, killed with formaline is used; 1 ml. of the preparation contains 2 billion microbial cells. The course of vaccination consists of 2 subcutaneous injections of 1 ml. each at intervals of 7-10 days. Immunization secures the creation of relative immunity for a period of 4-6 months. Revaccination is carried out in case of epidemic indications after every 4-6 months. The storage period of the preparation is 12 months. The adopted vaccine exerts moderate systemic and local reactions and is protecting 16-46% guinea pigs, infected subcutaneously with a virulent culture (96-100% of the control animals died). The vaccine precipitated with alum is protecting 80-100% guinea pigs under the same conditions. By increasing the dosage and number of injections, the results are improved. When the vaccination dose is increased to 8-12 billion microbial cells, the efficiency of the vaccination is increased; the results appeared to be even better upon revaccination (8,15,16,36,37).

Further research upon improvement of anti-plague vaccine in the USA is being carried out for obtaining the most effective antigens, representing individual fractions of killed cultures of the plague bacilli. It is assumed that a relatively low reactivity of these preparations with high immunogenicity will make it possible to change to multiple vaccination, which in turn will secure a strong immunity, preventing disease from aerogenic contamination.

Meyer succeeded in isolating 7 antigens from the plague bacillus, three of which possess, according to his data, important immunologic significance. Fraction I (F-1), representing water soluble protein fraction obtained from plague cultures, treated with acetone plays the most important part in creating immunity against plague; this fraction can be obtained in a crystalline form.

Fraction I, in a dose of 0.1 mg., protects white mice from 100 lethal doses of a virulent culture and possesses immunologic properties for rats, rabbits and monkeys. The immunogenic properties of F-1 become apparent in the formation of antibodies (agglutinins, hemagglutinins, complement-fixing, neutralizing). A twofold immunization of a person with 3 mg. of the preparation secures the appearance and significant increase of antibodies in man; 0.5 ml. serum of the immunized person protects the white mouse against 100 lethal doses of the virulent plague culture.

Meyer assumes that a condition necessary for the efficiency of any anti-plague vaccine, independent of whether the vaccine is live or killed, seems to be the presence in it of a sufficient amount of fraction I. In his opinion, strain EV contains comparatively little

fraction I and, therefore, appears as a relatively weak antigen; however its immunogenic properties can be increased by increasing the number of vaccinations. Meyer calculates that any anti-plague vaccine, live or killed, but containing 2-3 mg. of fraction I, will cause immunity in 50% of the persons vaccinated; to increase the intensity of immunity, revaccination after 3-6 months is necessary; the efficiency of vaccination is increased when the preparation is used in a water-oil emulsion.

In addition to fraction I, Meyer has isolated a second, toxic fraction representing a protein, but has not obtained it in a pure state. Upon parenteral administration of the toxic fraction in animals shock reactions develop, and death occurs in mice and guinea pigs; this fraction causes the formation of an antitoxin in blood which, however, does not protect other animals from the action of the toxin. The toxic fraction, according to Meyer, is contained in significant amounts in strain EV, causing its reactivity (38).

In March-April 1955 Ehrenkranz and Meyer (30) announced their comparative studies of three immunizing preparations for protection from the pulmonary form of plague. Authors of experiments with monkeys compared live (strain EV-76) and killed formalinized vaccines (strain Irek), and also fraction I which was extracted from plague microbes, strain Irek, killed with acetone and dried; afterwards this extract was precipitated and crystallized by the method of Beiker. Finally, in the observations carried out it was established that, with a preliminary immunization of monkeys with three preparations in doses adopted for humans, a weak protection is created against aerogenic contamination with  $10300 \pm 1100$  virulent plague microbes. By increasing the immunizing dose of vaccines, the survival percentage of contaminated monkeys increases, but insignificantly. Much better results were obtained by immunization with moderate as well as with massive doses of the vaccine, administered in manifold injections over a longer period of time. In these cases, immunization by any of the three vaccines secured 100% protection of monkeys from aerogenic contamination by  $10300 \pm 1100$  virulent plague microbes. Any difference in the quality of the preparations tested was not observed. It should be emphasized, especially, that the application of massive doses of antigen appeared to be possible only on condition of its thorough purification and concentration (30).

Silverman, Kaioshi, Kigushi and Meyers (40), obtained in 1954, a protective antigen against the plague microbe grown on a synthetic nutrient medium, consisting of pure amino acids and other ingredients. The filtrate of the plague culture obtained, in which fraction I and other antigens were found, after being treated with alum, was repeatedly introduced into white mice (the total amount of antigen introduced reached 5-10 ml. As a result, 60-100% of immunized animals were found

resistant to 100 lethal doses of a virulent plague culture, administered subcutaneously.

In England, as well as in the USA, vaccination against plague is performed on epidemic indications, whereupon, for this purpose a killed vaccine is supplied, containing 2 billion microbial cells per ml. The dose is 0.5-1 ml. subcutaneously, with an interval from 1-3 weeks. The duration of immunity is 3 to 12 months; revaccination is also carried out upon epidemic indications after 6 months with a single injection of the preparation (1 ml.) (20).

In Switzerland a killed anti-plague vaccine is used (1 ml. contains 2 billion microbial cells); twofold subcutaneous vaccinations in a dose of 0.5 ml. with an interval of 8 weeks; revaccination after 6 months with the same dose (21).

In France, for immunization of the personnel of the armed forces and of the population, a killed formalinized vaccine is also used (2 billion microbial cells per 1 ml.), it is introduced subcutaneously three times (in case of severe epidemics) in doses of 2-2-4 ml. with 3-5 day intervals (30).

For immunization of the population in Madagascar, Senegal, Congo, and South Africa, as well as in Tunisia and Algiers, a live vaccine from an attenuated strain EV (Girard, Robik) is prepared in France (Pasteur Institute). This vaccine is applied once, and it is not inferior in its immunologic efficiency to the killed vaccines, but, in the opinion of many authors, excels them; at the present time, it is prepared in the dry state which significantly increases its storage time (37,41).

According to data of Andrews, live vaccines lower morbidity in endemic foci, amounting to as much as 4-5 fold in times of epidemics. Otten, Girard and Robik (37) consider that vaccinations with a live vaccine in areas of epidemics, without carrying out other anti-plague measures, have lowered morbidity from plague 8-10 fold. Nevertheless, a live vaccine from strain EV, as well as other live vaccines, because of the danger of strain reversion, insufficient efficiency in pulmonary infection, and significant reactivity, has not been applied either in European countries or in America.

In the opinion of many specialists, the existing live and killed anti-plague vaccines do not create sufficiently strong immunity against the pulmonary form of plague, i.e., they do not protect from aerogenic contamination, and consequently, do not satisfy the demands required of the preparations intended for protection from a bacteriologic weapon. However, vaccination in case of epidemics is considered compulsory, since, by decreasing morbidity from bubonic plague,

it decreases at the same time the number of cases of secondary-pulmonary plague, which decreases the possibility of the development of epidemics of the pulmonary form of the disease (37).

Tularemia. The causative agent of tularemia, like that of the plague microbe, belongs to the probable agents of bacteriologic warfare. Although fatality from tularemia is not high, and there are highly effective therapeutic remedies securing the successful treatment of this disease, immunization against tularemia, reliably preventing the disease, might find a wide application under conditions of bacteriologic war (see chapter IV).

In "The Control of Communicable Diseases in Man", published in 1953 by the American Public Health Association, it is indicated that immunization against tularemia in the USA is not carried out (42); also there are no data in regard to vaccination of the personnel of the armed forces against this infection. However, according to other sources, there is in the USA a so-called Foshay vaccine, applied upon epidemic indications for prophylaxis from tularemia (43).

This vaccine represents a tularemia culture, treated with nitric acid, washed, and suspended in a 0.5% solution of phenol. The preparation contains up to 24 billion microbial cells per ml. and is injected three times at 1 day intervals; the duration of the effectiveness of the vaccine is one year, at a temperature of 4°. According to general data, vaccination of 2145 people (from 1932 to 1941) did not guarantee against disease, but somewhat decreased the number of diseased and contributed to lighter course of disease (26).

In 1950, Kadull et al. (44) announced the production of a phenol-treated and acetone-extracted vaccine, as well as the vaccination of 809 persons with these preparations. In 20% of the vaccinated persons within a period of 2 months, a positive allergic reaction appeared, and in 48 persons, out of 207 examined, agglutinins in the blood were detected. Out of 72 vaccinated, who worked every day with virulent tularemia cultures, 22 persons became sick. The authors assume that persons working with this infection should be immunized against tularemia.

Within the past 5-6 years in the USA a series of experimental works on obtaining different soluble antigen fractions from tularemia cultures (chemical vaccine) has been published. By immunizing white mice with a soluble antigen, a specific resistance appears in them, apparent already 24 hours after immunization and not accompanied by an accumulation of specific antibodies (45).

Indications of the application of live vaccines against tularemia, in the USA, as well as data on the availability of analogous



preparations in other countries, cannot be found in the published literature.

Brucellosis. According to the opinion of American specialists, the causative agent of brucellosis can be applied with great probability as a bacteriologic weapon (see chapter IV). Considering the absence of highly effective remedies for treatment of brucellosis, it can be assumed that vaccine prophylaxis might play an important role in combating this disease in peace time, as well as, particularly, under conditions of bacteriologic war. However, in the USA, vaccination of the people against brucellosis is not carried out and is considered "impractical". No data on anti-brucellosis vaccine, applied for prophylaxis against this disease in humans, could be found in the official manuals available. However, in the special literature, there are some indications of attempts to make, in the USA, a live vaccine against brucellosis. In 1953 there was obtained a vaccine strain, Br. melitensis (having dependence on streptomycin), producing a rather intensive immunity in white mice toward experimental infection (46).

Along with this, in veterinary practice in the USA, active immunization with live vaccines is used with a dry vaccine prepared from bovine strain No. 19, with which calves are vaccinated (47).

According to the data of American manuals, killed vaccines do not give positive results for prophylaxis of animals against brucellosis (48).

Nevertheless, anti-brucellosis vaccines are still used in medical practice in the USA, not for prophylactic, but rather for therapeutic purposes.

For this purpose, a bivalent or monovalent killed vaccine from microbes of the bovine, caprine, or porcine type is applied.

The wide experience in the USSR with the successful vaccination of humans with a live vaccine made from bovine strain No. 19 is elucidated in foreign literature.

Anthrax. Along with the plague microbe, the causative agent of anthrax is considered as one of the most probable agents in bacteriologic warfare. The extremely high fatality observed in the pulmonary and septic forms of the disease, determines the leading role of prophylactic measures in the general preventive system against anthrax. In connection with this, vaccination against anthrax, securing protection of humans against aerogenic contamination, might appear as an important measure for protection in bacteriologic warfare. Nevertheless, vaccines against anthrax are finding a wide application only in veterinary practice at the present time in the USA.

Immunization of animals in the USA is accomplished:

- with "bacteria against anthrax", representing a suspension of killed bacilli in formalin solution; it is recommended for vaccination of horses, large and small livestock;

- with spore vaccine No. 3 - live attenuated spore culture, treated with saponin; it is recommended for vaccination of large livestock;

- with a "spore vaccine No. 4", which is prepared from a live culture of a Bact. anthracis strain with a proved antigenity; the washings from agar cultures are suspended in a buffer solution;

- with a "special spore vaccine No. 4" which is applied, together with immune serum, for a fast liquidation of infection in outbreaks of anthrax (47).

In recent years, in connection with working out problems of anti-bacteriologic protection in the USA, work has been started for the creation of an anthrax vaccine for people, preventive against aerogenic contamination. The working out of the new vaccine is based on obtaining anthrax antigens from edematous tissues of the infected animals or from cultures of the causative agent grown on special media.

A special synthetic, nonprotein medium was developed for cultivation of the anthrax bacillus and for obtaining antigens from it. This medium consists of 17 aminoacids, inorganic salts, guanine, uracil, thiamine, glutamine, glucose and sodium bicarbonate. As a specific anthrax antigen ("protective antigen"), the filtrate from the culture of the causative agent cultivated for 20 hours at a temperature of 37° on the medium indicated above, is used; it is filtered through ultra-porous glass filters. The lyophilized preparation is preserved for several months. The concentration of antigen is attained by precipitation with aluminum potassium alum, which is done immediately after filtration.

The immunologic efficiency of the preparation was first tried out on rabbits and guinea pigs, later - on monkeys.

It was established that, in rabbits, the antigen produces strong immunity, protecting them against 100 LD<sub>50</sub> and maintaining this on a high level for more than 3 months. Experiments on immunization of guinea pigs showed that 2-3 fold vaccination secures survival of animals after intradermal inoculation with 200 LD<sub>50</sub>.

In experiments with monkeys it was established that immunization with two injections (subcutaneous) in doses of 0.5 and 1 ml. with an interval

of 2 weeks, creates strong immunity, protecting monkeys from intramuscular infection with 50,000 to 100,000 spores. Eight monkeys were immunized subcutaneously twice with a dose of 1 ml. of the preparation, with an interval of 16 days. Six days after the second injection, 4 immunized and 4 control monkeys were aerogenetically infected with 39,000-82,000 anthrax spores. The control animals died from systemic infection, whereas the immunized ones survived; vaccinated monkeys tolerated aerogenic infection even with 890,000-3,000,000 anthrax spores. In this way the validity of the preparation for protection against aerogenic infection was brought to light (49,50,51).

After this, tests were tried out on humans. A group of 55 volunteers received 2 injections each at a dose of 0.5 ml. basic antigen with an interval of 2 weeks. No significant systemic reactions were observed, and only in 3 persons a slight painfulness appeared at the site of injection after 24 hours.

Afterwards, the immunization program was broadened to conform to a large group. Vaccination was carried out three times with a dose of 0.5 ml. each with an interval of 2 weeks; revaccination was carried out after 6 months with a dose of 0.25 ml. A total of 1936 injections were made in 660 persons, whereupon systemic reactions were observed in 0.7% of the cases and significant local reactions - in 2.4%; on revaccination of 445 persons, the number of reactions increased correspondingly to 1.3 and 2.7%.

In general it was established that humans tolerate the administration of antigen well; serious reactions are absent, and moderate local reactions are observed seldom.

Cholera. The causative agent of cholera is regarded as a possible agent in bacteriologic warfare (see chapter IV). The vaccine adopted in the USA against cholera represents a suspension of killed, heated cholera vibrios, containing 8 billion microorganisms per 1 ml.; the preparation can be kept for 18 months. The basic course of immunization consists of two subcutaneous injections - 0.5 and 1 ml. each, with an interval of 7-28 days. According to the data of the International Quarantine Commission, immunity against cholera develops already 6 days after the first injection; however, it appears to be relative; the duration fluctuates from 6 to 12 months. The reactivity of the vaccines is insignificant and severe reactions are not observed. Revaccination is carried out every 6 months in a dose of 1 ml.; it is calculated that it is effective if it is carried out for 4 years according to the basic course of immunization.

Vaccinations against cholera are carried out in the army and among the civilian population, on epidemic indications, especially in case of trips to areas, where the morbidity is endemic, or in case of

an epidemic.

Also the application of the causative agent of cholera by the enemy, as a bacteriologic weapon appears as an indication for mass immunization (2,8).

The causative agents of rickettsiosis - epidemic exanthematous fever, Q-fever, Rocky Mountain spotty fever and tsutsugamushi fever, according to the data of American authors, may be used as bacteriologic weapons.

Therefore immunization against these infections along with prevention of "natural" morbidity may pursue the goal of protection of the population and the armed forces from bacteriologic attack.

Exanthematous fever. Exanthematous fever in the USA belongs to the category of infectious diseases, active immunization against which is carried out on epidemic indications. These indications, at the present time, are mostly trips to countries where exanthematous fever is endemic. Epidemic disturbances inside a country, arising from a "natural" epidemic or bacteriologic attack, are also considered as indications for carrying out vaccinations. These indications also apply to the personnel of the armed forces in the USA.

Cox formaldehyde-killed vaccine, adopted in the USA, represents a suspension of killed *Rickettsia promazeki*, cultivated on developing chick embryos (1,2,8,15,16).

The course of immunization consists of two 1 ml. injections with an interval of 7-10 days. As a result, a relative immunity is created which remains from 6 to 8 months, reaching its maximum after 3 months. Revaccination is carried out after every 6 months, as well as in case of epidemic indications. It is proved that single immunization, carried out even 5 years after immunization, causes sufficient accumulation of antibodies in vaccinated persons. The vaccine described is not preventive against endemic or murine typhus (tabardillo), tsutsugamushi fever and other rickettsioses. The preparation is valid for 12 months.

Exact evaluation of the epidemiologic efficiency of the vaccine is not given; however, in general, it is admitted that it decreases morbidity and alleviates the course of exanthematous fever. During World War II, 64 cases of exanthematous fever were registered in the American army; they all took a light course without fatalities (52).

The disadvantages of Cox vaccine (it does not guarantee from infection) prompted American scientists to find more effective, prophylactic preparations against exanthematous fever. At the present

time in the USA the working out of a dry, live anti-exanthematous fever vaccine from strain E has been successfully completed.

The Spaniards, Clavero and Gallardo (53,54) in 1943, by transferring Promazek's rickettsia on chick embryos, observed an accidental change of its properties: the strain sharply decreased in virulence. The authors designated this as strain E (in honor of Spain - Espanha). Working in Spain, they carried out extensive investigations on the harmlessness and immunogenicity of this strain. They vaccinated 2242 volunteers. However, in the light of contemporary data, the results obtained have comparative significance, because too low a dose (less than one minimal infective dose) was used for immunization. Further investigations, since 1946, were carried out jointly with Fox (USA). In 1951, work was started with volunteers in the USA, including approximately 200 persons. As a result of these investigations, the minimal vaccination dose of strain E rickettsia for humans was determined (3.5 - 4 log EID) and the direct relationship between the maximal serologic response (reaction of complement binding, neutralization reaction) and the immunizing dose was established; it was shown that an immune response in vaccination was caused by doses equal to log EID of 4 or more; thereby a sufficiently strong immunity, protecting the vaccinated persons against a virulent culture of Promazek's rickettsia, is maintained for at least 2 years after a single injection. The authors established also that the most effective method of injection is the intradermal or intramuscular (subcutaneous - is worse).

Although immunity, caused by vaccine E with a single injection is weaker than natural immunity developing after disease, however, compared with immunity caused by Cox vaccine, it appears to be much stronger. This was proved in volunteers on the basis of serologic reactions as well as in direct infection experiments with a virulent culture of Promazek's rickettsia.

The reactivity of the new vaccine depends on the dosage. Strong reactions, according to authors, were not observed; however, moderate reactions (local and systemic) were observed rather constantly.

Beginning with 1953, studies on the vaccine were carried over into a wide epidemiologic experiment in an area in Peru endemic in regard to exanthematous fever; 19,000 persons were under observation. These trials basically confirmed the data obtained in the USA. However, it is necessary to indicate, that although the authors noted a relatively low reactivity of the vaccine according to their own data, in 1% of all vaccinated persons reactions were observed, representing a true disease with exanthematous fever; it is characteristic that in the group of vaccinated persons older than 30 years, the percentage of such disease reached 4-5. It was also established that the lyophilized vaccine obtained can be successfully preserved for 8 months

(period of observation) (53,54,55,56,57).

The epidemiologic experimentation was continued in 1955 in Peru (Andina area) on 10,000 volunteers. Results of this trial will make it possible to answer the question concerning the epidemiologic efficiency of the new vaccine.

In England and Switzerland, a complex vaccine against epidemic and murine exanthematous fever, prepared on chick embryos and representing a suspension of killed (with formalin) Promazex's and Mooser's rickettsiae is supplied. The primary vaccination is carried out with a three-fold injection of 1 ml. each with intervals of 8-10 days. Revaccination is done with 1 ml. of the preparation after a year; in case of unfavorable epidemiologic conditions, revaccination is done after 3-6 months (20,21).

In France, contrary to other countries, a formalin killed vaccine is used, prepared according to the method of Durand and Giraud from light-weight white mice. The primary vaccination is done with three injections of 1 ml. each, after 8 days and revaccination - after a year with 1 ml. Along with this, the exanthematous component is included in the associated vaccine developed in France, against typhoparatyphoid fevers (TAB), diphtheria and tetanus.

Q fever. A highly effective vaccine against Q-fever has been developed and released in the USA; the preparation secures the creation of strong immunity in vaccinated persons, and is overcome only with a very high infectious dose; it is characteristic that the disease in such case takes a light course.

The vaccine is produced on egg-yolk sacs of developing chick embryos; the suspension is inactivated with a 0.25% solution of formalin. Three subcutaneous injections of 1 ml. each are given at an interval of one week. Revaccination of 0.5 ml. is done every 4 months. Two to three months after revaccination the complement binding reaction titre increases from 1:20 to 1:40. Immunization is carried out upon epidemic indications (58,59).

Recently, in the USA, soluble rickettsia antigens were investigated. To these belong rickettsia antigens of epidemic and murine exanthematous fever, Rocky Mountain spotted fever, Marseille fever, and rickettsialpox. In 1956, publication of Colter, Brown et al. (60) appeared on soluble antigen bound with the causative agent of Q-fever, which can be separated from rickettsiae by means of ultra sound vibrations. The authors prove that solutions, containing antigen, cause a formation of antibodies in guinea pigs and create in the animals a relative immunity against infection with a virulent strain.

Rocky Mountain spotted fever. Two vaccines against Rocky Mountain spotted fever are used in the USA; one is prepared from phenolized tissues of infected ticks (*D. andersoni*), the other - from rickettsiae, cultivated on chick embryos. Both vaccines are prepared in a laboratory located in the state of Montana, and appear to be rather effective preparations. The immunization scheme is: three injections, 1 ml. each, subcutaneously or intramuscularly after 7-10 days with revaccination after one year.

In children, a definite immunity against the most virulent strains is created. In adults, the strength of immunity is not as great; morbidity among vaccinated persons is observed; however, the clinical course of the disease is alleviated and fatality is decreased.

Vaccinations are carried out upon epidemic indications (61,62,63).

Tsutsugamushi fever. It is established in the USA that vaccination against tsutsugamushi creates a weaker immunity than having the disease. Immunization with killed vaccines gives unsatisfactory results, whereupon only a comparative type-specific immunity towards a given strain is produced. American authors, at present, consider the making and application of a polyvalent vaccine a difficult task.

In view of the lack of an effective vaccine under especially unfavorable conditions (endemic areas), the use of the "minimal infection method", by an intradermal injection of a minimal infective dose of the virulent rickettsiae followed by chemoprophylaxis with chloramphenicol, is recommended. After 10-12 days, an increase in temperature is observed in the persons vaccinated; if the duration of temperature reaches 36-48 hours, antibiotic treatment is carried out.

This prophylactic method secures immunity from 1-2 months up to one year (62,64,65,66).

Causative agents of virus diseases - yellow fever, psittacosis, equine and Japanese encephalomyelitis, are considered by American specialists as probable agents of bacteriologic warfare (see chapter IV). The role of vaccine-prophylaxis in the general system of combating these diseases is especially great in connection with the fact that an effective treatment of these infections (except psittacosis) has not been developed, and fatality is rather high.

Yellow fever. Yellow fever in the USA and European countries belongs to the diseases against which immunity is carried out on epidemic indications, especially in case of trips to endemic foci. The following countries in Central America are considered by the USA to be endemic areas of yellow fever: Costa Rica, Cuba, Nicaragua, Panama,

Trinidad, and also the Bermuda Islands; in South America - all countries, except Argentina, Chile, Paraguay and Uruguay; in Africa - a significant part of the territory (mainly Western and Central Africa). Vaccination is given 12 days before arrival to the area, and after returning to the USA. Besides, in many countries - as India, Ceylon, Pakistan, although yellow fever is not observed, all conditions exist for dissemination of this disease because of the presence of the mosquito - carrier. Therefore, the above mentioned countries observe a strict quarantine, and compulsory vaccination against yellow fever is required from all who enter (8).

Naturally, that the possibility of the application of a yellow-fever virus as a bacteriologic weapon is also considered as indication for mass immunization.

For vaccination against yellow fever in the USA, a highly effective live vaccine, strain 17D, developed and proposed by Teylor, is used. The preparation is made from an attenuated virus strain of yellow fever, obtained by means of prolonged cultivation in tissue cultures. Accumulation of the vaccine strain takes place in developing chick embryos with subsequent drying and keeping in a dry state at a temperature below zero until it is used. This preparation can be kept for two years.

After immunization, the organism is protected for at least 6 years. Work is being carried on for improving the vaccine in order to increase the duration of immunity after vaccination for 9 years.

Vaccination is carried out with a single subcutaneous administration of 0.5 ml. of the preparation, diluted 1:10. In endemic areas, revaccination is performed after 6 years with a subcutaneous injection of 0.5 ml. of the vaccine (8,15,16,24).

In the past few years in the USA, a combined live vaccine against yellow fever and natural small-pox has been developed; it is introduced into the organism by means of skin scrapings. Investigation of this vaccine showed that immunity against yellow fever develops in the vaccinated persons slower than against small-pox, but after several months it develops in 92-95.6% of the persons subjected to vaccination. Furthermore, however, it was established, that subcutaneous vaccination against yellow fever produces stronger immunity, than cutaneous administration. This, seems to be one of the reasons for the fact that, at present, vaccinations against small-pox and yellow fever in the USA are carried out separately, at an interval of 3 weeks. However, in Africa, under definite conditions (epidemics), successful immunization of the local population has been performed with an associated vaccine (24,67).

In England, same as in the USA, live vaccine from strain 17D is



produced in chick embryos. Experimental immunization with this vaccine secures the protection of animals against 500 LD<sub>50</sub>. The immunity is produced in 10 days and is maintained for 6 years. Injections are carried out subcutaneously and reactivity is insignificant. The vaccine is kept in the dark at a temperature of 1-2° for 3 months; at a lower temperature, the storage period may be increased. At 20° the vaccine loses its activity in a period of few days. In England, at the present time, preparations have been developed, which can be stored for 12 months (20).

In France and its African colonies, along with vaccines from strain 17D, a live vaccine from the attenuated neurotropic strain "Dakar" has been developed and used; the virus for production of the vaccine is accumulated in the brain of white mice. This vaccine, it appears, is distinguished by a greater immunogenicity; however, it produces reactions. Upon immunization of the population of Eastern Nigeria with the neurotropic strain, 3-4 persons out of every 1000 vaccinated (mainly children) became sick with encephalitis, which ended fatally in 40% cases (24,68,69).

Psittacosis. In the USA there is a vaccine against psittacosis; it does not appear on the market and is produced in small amounts for special purposes. It is prepared from egg yolk sacs of infected chick embryos; the emulsion of the sacs is treated with formalin and afterwards extracted with ether. There are no convincing data at the present time on the effectivity of the vaccination in infections with different strains of the virus.

Vaccination is carried out three times with 1 ml., with a one week interval, and revaccination - with 0.5 ml. after every 6 months. Injections are carried out in case of epidemic indications.

Eastern and western equine encephalomyelitides. In the USA, for veterinary purposes, vaccines against eastern and western equine encephalomyelitides are developed and applied. These vaccines represent formalinized tissue extracts from chick embryos, infected with the virus of encephalomyelitis (47).

The vaccines proved to be extremely effective in immunizing horses justifying their use also in humans. For this purpose, in the USA a bivalent vaccine against eastern and western encephalomyelitides was prepared. It was made by means of a separate cultivation of viruses of eastern and western encephalomyelitides on chorioallantois membranes of chick embryos, followed by inactivation with formalin. The monovaccines obtained in this way, were afterwards mixed in equal amounts, and a bivalent vaccine was obtained. The infectivity of the extracts before treatment with formalin was  $10^{-7}$  for the eastern strain and  $10^{-6}$  for the western.

Upon approval the vaccine was administered to humans (100 persons) two times intramuscularly at a dose of 2 ml. at an interval of 1 week. Systemic and local reactions were very weak; the vaccine caused an intensive production of virus-neutralizing antibodies, whereupon the minnogenity of the western strain appeared to be higher; high antibody titers appeared after 7 days from immunization with western variant, and after 2 weeks from immunization with the eastern variant.

The authors recommend vaccine against equine encephalomyelitides for immunization of humans who are "often subjected to the action of the virus under laboratory or field conditions" (62,71,72).

Venezuelan equine encephalomyelitis. According to data of American authors, active immunization against this disease seems to be an effective means of prophylaxis for persons subjected to danger of infection. In the USA the vaccine is supplied for the army and is prepared from a culture, grown on chick embryos.

Vaccination is carried out with two injections, 1 ml. each, at an interval of one week; revaccination - after every 6 months. Immunization is carried out on epidemic indications (62,73).

Japanese encephalitis. In the USA, army vaccination against Japanese encephalitis is applied on epidemic indications (trips to endemic areas). The vaccine is made from a virus, cultivated on developing chick embryos and representing a suspension of homogenized chick embryos. The vaccine is very unstable and must therefore be kept in a dry state until the time of application.

The development of the vaccine is not yet completed, and there are no basic data as yet on its efficiency.

During the war in Korea, vaccination was carried out in 1951 only, later on it was abolished because of inefficiency (62,74,75).

Botulism. Botulism toxin, along with the causative agents of plague, anthrax and many other diseases, is considered as the most probable agent of bacteriologic war. Taking into account the extremely high fatality from botulism and the absence of effective antibiotics and chemotherapeutic agents for treatment of this disease, as well as the very strong immunity, which is created in vaccinated persons, it must be admitted that immunoprophylaxis appears to be the basic means of combating mass-morbidity from botulism in case of bacteriologic warfare. Thereby it is necessary to emphasize that a systematic vaccination and revaccination against botulism makes it possible to produce in the human and animal body a "protective" level of antibodies, preventing massive infection with the toxin, making it possible, principally, by means of planned immunization, to protect

the armed forces and the population from this form of bacteriologic weapon.

In France, polyanatoxins of type A, B, C, D, and E are produced, representing a filtrate of broth cultures, treated with formalin.

The preparation is usually used therapeutically three times, in doses of 2 ml. subcutaneously at intervals of 8 days; before the basic course of treatment, preliminary 0.1 ml. injection of the anatoxin is given to determine sensitivity; in case of a strong local reaction, with a tendency toward necrosis, immunization is not carried out.

Indications for such a treatment may be light forms of botulism, continuing for several days without the appearance of the main symptoms, the same method can be used also in severe cases.

Vaccine-prophylaxis, in this case, is combined, as a rule, with serumtreatment (30).

In the USA, a combined anatoxin of type A and B is used for immunization of laboratory workers; one ml. of the preparation is introduced three times, subcutaneously, at four week intervals. After immunization, in 90% of all vaccinated persons, 0.02 AU is detected in one ml. blood, but in 30% - the antibody titer exceeds 0.1 AU. Guinea pigs with a titre of 0.02 AU or more tolerate 200,000 Dlm of the toxin; a content of 0.1-0.5 AU in the blood prevents the animals from 1,000,000 Dlm. After 6 months, the immunity decreases; therefore revaccination with 1 ml. is necessary (76,77).

Associated vaccines. The presence of a significant number of causative agents, which might be used as bacteriologic weapons, necessitates the creation of numerable vaccine preparations for prophylaxis of each of the infections and significant broadening of the scheme for routine vaccinations of the armed forces and population. However, the utilization of monovaccines for this purpose can extremely complicate the complete scheme of vaccination and make it practically impossible from the medical as well as from the purely organizational standpoint. A rational way out of this situation is the application of effective associated vaccines, which make it possible, with a minimal expenditure of work, to carry out mass immunization simultaneously against several infections. Naturally, that associated vaccines must possess a primary significance as a means of specific protection against bacteriologic weapons (4,5).

According to data published in foreign literature, systematic work for developing associated vaccines in the USA was not started until 1955, after a preparation against tetanus and diphtheria was produced.

For routine immunization of the personnel of the armed forces and of certain categories of the civilian population in the USA, the following is adopted:

- a complex vaccine against typhoid and paratyphoid fever A and B (1,4);

- a complex anatoxin against tetanus and diphtheria (1);

- an associated vaccine against typhoid fever, paratyphoid fever A and B, and tetanus; the preparation contains per ml., 1 dose of tetanus anatoxin, 1 billion typhoid and 500 million paratyphoid microbes; injections are made three times: 0.5 - 1 - 1 ml. at intervals of 4 weeks, and revaccination after one year (19);

- botulin anatoxin of type A and B (see chapter IV).

Besides, in pediatric practice, the following vaccines are applied:

- against diphtheria and whooping cough (for children under 10) (8).

To the associated vaccines used in the USA on epidemic indications, the following belong:

- a vaccine against natural small-pox and yellow fever (24,67);

- a vaccine against eastern and western equine encephalomyelitis (62,71,72).

Besides, recently an associated vaccine against yellow fever and dengue fever, prepared from attenuated strains of viruses has been developed and approved for humans (78). Preliminary positive results have been obtained with a test of an associated vaccine against diphtheria, whooping cough, tetanus and poliomyelitis (19).

From the associated vaccines prepared in England, besides vaccines against diphtheria and tetanus, diphtheria, tetanus and whooping cough, diphtheria and whooping cough and against typhoparatyphoid fevers (TAB) and tetanus, a complex vaccine against typhoid fever, paratyphoid fever A and B and Asiatic cholera deserves attention; injections are performed twice, at a dose of 0.5 and 1 ml. respectively at an interval of 7-28 days (20).

Original associated preparations, released in France, are:

- a vaccine against typhoparatyphoid diseases (TAB), tetanus and

diphtheria, adopted for routine vaccination in the army and navy;

- a vaccine against typhoparatyphoid diseases (TAB), tetanus, diphtheria and exanthematous fever, intended for vaccinations on epidemic indications;

- a vaccine against natural small-pox and yellow fever;

- a botulin polyanatoxin (types A, B, C, D, E) (30).

In Switzerland, vaccines adopted everywhere abroad, are produced (TAB - tetanus, diphtheria, TAB - tetanus - diphtheria, TAB - cholera) (21,79).

The problem of mass immunization of the population is solved in the USA and certain other countries, not only by producing complex vaccines, but also by the improvement of the technical means for carrying out the vaccinations themselves. In recent years, in the USA a half-automatic injector (spurting injector with many doses) was constructed, which is intended for introducing liquid vaccines, antibiotics and other therapeutic-prophylactic preparations under high pressure (without a needle) through the unbroken skin. In the beginning, the injector is loaded with the necessary amount of the preparation and after the first injection, it loads itself automatically. The testing of this new device in the experimental stage and on humans showed the possibility of applying it for administration (into the human body) of insulin, pituitrin, antibiotics and other preparations, different doses of which may be as much as 1 ml. or more. The tests with the spurting injector for mass immunization of anti-typhoid trivaccine, carried out in the army of the USA, confirmed its superiority over the usual injector in regard to less painful injection, as well as to speed of execution. The trial showed that by means of the device, more than 500-600 injections can be made in one hour. To disadvantages of the injector are: a slight trauma at the site of administration of the preparation, and also the impossibility of carrying out intramuscular injections and the introduction of viscous and oily suspension (18,80).

A review of the given materials makes it possible to draw many conclusions on the status of vaccine-prophylaxis as means of specific protection against bacteriologic weapons. The system of planned vaccination, adopted at the present time in the USA and other capitalistic countries, predicts the prophylaxis of "natural" infectious disease; this, however, does not secure the protection of the armed forces and of the population against infections, the causative agents of which might be used as bacteriologic weapons.

It is assumed that active immunization against infections, urgent from the military point of view, will be carried out on epidemic indi-

cations, i.e., after its application by the enemy as a bacteriologic weapon. For this purpose, in the USA, there are vaccines against plague, cholera, natural smallpox, epidemic exanthematous fever, Rocky Mountain spotted fever, Q-fever, yellow fever, equine encephalomyelitis, psittacosis and botulism of the types A and B.

In connection with this, in recent years in the USA, intensive work has been developed for the creation of new and improvement of existing vaccines controlled by the Ministry of Defense and Federal Agency for Civil Defense. As a result of these investigations, the production of highly prospective chemical vaccines against anthrax, plague and typhoid fever, as well as the successful development of a liquid vaccine against exanthematous fever of strain E, appeared. Extensive research in the field of vaccine-prophylaxis is in progress at the present time.

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## THE RAPID THERAPEUTIC SERA.

In connection with the widespread introduction into medical practice of antibiotics and chemical preparations possessing a high therapeutic effectiveness with respect to a considerable number of bacterial and rickettsial infections, the area of application of specific therapeutic sera has shrunk considerably during the last few years. Presently, however, serotherapy and serum prophylaxis preserve their significance in the struggle with toxico-infectious [botulism, tetanus, gaseous gangrene, diphtheria], and virus [measles, encephalitis] diseases. Serotherapy did not lose its significance in the treatment of serious forms of the plague and malignant anthrax as well as for the prophylaxis of these diseases.

At the present time, specific therapeutic sera are considered, therefore, as an effective means in the fight against certain infectious diseases, and are included in the arsenal of special defense agents against bacteriological weapons. From this point of view, therapeutic sera against the plague, malignant anthrax, tetanus, encephalitis, and particularly botulism, for the treatment and special prophylaxis of which there are currently no other effective means, deserve special attention.

The importance of seroprophylaxis and serotherapy can increase significantly in cases in which the pathogens of infectious diseases prove to be resistant to the normally applied antibiotics and chemical preparations; this can occur under conditions of bacteriological warfare.

A short characterization of therapeutic sera manufactured by firms of the main capitalist countries [the USA, England, France, and others], as well as elucidation of basic principles regarding their therapeutic and prophylactic application abroad is presented in the current section.

**ANTITOXIC SERUM AGAINST BOTULISM.** At the present time, a concentrated anti-botulin serum, available both in the native as well as in concentrated forms, is manufactured in the USA, England, and France,

whereupon in the USA and England, a bivalent preparation [types A and B], and in France, a pentavalent one [types A, B, C, E, and H] are produced. The sera are prepared by hyper-immunization of horses with the appropriate type of the botulin anatoxin. The titer of currently manufactured preparations is not reported.

The medicinal antitoxic serum against botulism is applied abroad both for therapeutic as well as for prophylactic purposes. It is emphasized that its utilization is of great value prior to the manifestation of the disease's symptoms. The serum's therapeutic effectiveness decreases when the appearance and development of the clinical picture of botulism have already set in.

In the USA, 2,500 AE of the bivalent [A and B] serum [1] are administered subcutaneously for prophylaxis. In England intramuscular introduction of the preparation in a dose of 10-20 ml is recommended. It should be mentioned that in England, a series of therapeutic-prophylactic centers functions, where at any time of the day one can obtain anti-botulin serum [2].

In France, when poisoning from pork meat is suspected, for prophylaxis, only type B serum in the amount of 20 ml is applied. When infection through other foodstuffs and canned foods is suspected, A, B, and C type sera in various doses are utilized; when infection from fish products is suspected, E type serum is incorporated. Inasmuch as in France no cases of type D botulism disease were observed, a similar kind of serum is administered only when specific indications are manifested [4].

Anti-botulin serum is the sole effective means for the treatment of botulism. The effectiveness of serotherapy depends closely on how soon after contraction of the disease treatments are begun: the earlier serum is administered, the higher the therapeutic effect. In the USA, intravenous introduction of the bivalent [A and B] serum in a dose of 10,000 AE [1] is recommended; in the absence of an effect, the serum administration is repeated. Lekh [3] considers it as imperative to increase the single therapeutic dose to at least 50,000 AE and to introduce it intramuscularly. In England, for treatment of botulism, intramuscular introduction of a 30-50 ml bivalent serum dose, and repetition of such injections in the absence of an effect within 8-12 hours is recommended. In France, when taken ill by the disease associated with food consumption of pork, for the performance of bacteriological diagnosis, 40 ml of type B serum are introduced daily up to the manifestation of the clinical effect. In infections associated with other foodstuffs, a polyvalent serum of types A, B, C and E, containing 40 ml of each, i.e. a total of 160 ml is administered. In the first injection, one half of the serum dose is introduced intramuscularly, while the

other half is administered subcutaneously; later on, the serum is injected only subcutaneously [4]. Prophylactic and therapeutic serum doses are presented in table 7.

Table 7

		USA	England	France
Prophylactic dose		2,500 AE [A and B]	10-20 ml [A and B]	20-80 ml [A,B,C,E,]
Therapeutic dose	Daily dose [first few days]	20,000- 100,000 AE [A and B]	60-150 ml [A and B]	160 ml [A,B,C,E,]
	Dose for the entire course of treatment	Until an effect is produced		

In the opinion of American authors [5], the anti-botulin serum, although effective against all five varieties of botulism, is nevertheless usually administered too late for manifestation of a therapeutic effect; as a result, botulism's mortality rate, until now has comprised 65%.

**ANTIBACTERIAL SERUM AGAINST THE PLAGUE.** A therapeutic antibacterial serum against the plague is made in the USA, England, France, and other countries; it is produced initially by hyper-immunization of horses with anti-pestilential vaccine, and afterwards by the virulent *B.pestis* culture. It finds application both for prophylaxis and treatment of the plague.

In the opinion of American authors [6], persons coming in contact with patients suffering only from the pulmonary [primary and secondary] form of the plague should be subjected to sero prophylaxis; persons coming in contact with patients suffering from the bubonic form are not subject to sero prophylaxis. For prophylaxis, the serum is introduced intramuscularly in a dose of 10-20 ml. Such a dosage was also adopted in France. Wu Lien-te [cited by Politzer (6)] recommends intramuscular administration of 100 ml of serum for those coming in contact with patients suffering from the pulmonary form of the plague, increasing this dose to 200 ml for those in great danger of infection; and according to his data, administration of 40-50 ml of serum is sufficient for the early performance of sero prophylaxis.

In spite of the demonstrated effectiveness, seroprophylaxis, at the present time, does not find wide application in the fight against the plague in endemic disease centers abroad. The reasons for this state of affairs appear to be, first of all, the high price of the serum, and secondly, serum reactions [6]. Departing from this, and taking into account also the prophylactic effectiveness of sulfanilamides, and in view of their relatively low price as well as length of preservation, the international committee of experts on plague control of the World Organization in Care of Public Health [second session of the 1952 congress in Bombay], recommends the application of sulfanilamides [sulfadiazine, sulfathiazole, and others] in a 3 gram dose per day for six days for plague prophylaxis for persons coming in contact with patients suffering both from the pulmonary as well as the bubonic forms of the disease.

In spite of the high prophylactic effect of antibiotics [streptomycin at the rate of 1 gram per day in the course of five days], special mass prophylaxis of the plague with these compounds is not being performed in colonial countries, in view of their relatively high cost [6].

For treatment of the plague, the anti-plague serum was first applied by Yersin in 1896. It remained for a long time the sole specific therapy for this disease, giving a positive effect in the treatment of bubonic plague.

Subsequently, sulfanilamides were successfully applied in the therapy of this form of the disease. At the present time the most effective means for treatment of all forms of the disease is acknowledged to be streptomycin which has pushed back into the background both sulfanilamides as well as the serum.

Opinions regarding the serum's effectiveness and indications for its application differ widely at the present time. Thus the Indian Commission on Plague Control points out that the anti-plague serum produces an effect, but it is lower than that of antitoxin sera [for instance, of anti-diphtherial serum]. In the opinion of Sokkhei, a daily dose of 40 ml [20 ml intravenously and 20 ml intramuscularly, in the course of five days appears to be sufficient in the treatment of serious cases of bubonic plague. Le Gall and Pollitzer [6] recommend a daily administration of 40-60 ml within a 24 hour period. Other authors increase the daily dose for adults to 100 ml, and for children to 50 ml. Concurrently an opinion is prevalent that in very rare cases, serotherapy can save even patients afflicted with the primary pulmonary form of plague, a fact about which Le Mauné and a number of other authors have reported. Two cases of curing the septic form of plague with serum [cited by Pollitzer - 6] are also described in foreign world literature.

When serotherapy is combined with sulfanilamide [sulfadiazine, sulfathiazole] treatment, the effect of treating the bubonic form of plague is enhanced.

Conclusive data on curing by this means a considerable number of patients afflicted with the primary pulmonary form of plague [6] is available.

In general, experience of several years standing regarding the application of anti-plague serum indicates that a positive therapeutic effect results only in the treatment of the bubonic form of plague under conditions of early initial start of therapy. Serum treatment of the pulmonary and septic forms of the disease, as a rule, prove to be unsuccessful.

ANTIBACTERIAL SERUM AGAINST MALIGNANT ANTHRAX. Therapeutic serum against malignant anthrax is obtained by the hyper-immunization of horses or bulls [in Switzerland] with Bact. anthracis cultures, whereby at the beginning of the immunization a vaccine strain is applied, followed by a virulent one. In the majority of foreign countries [the USA, England, France] the serum is produced both in the native as well as in the form of a purified and concentrated preparation [3].

In view of the rarity of cases among people taken ill by malignant anthrax, prophylaxis is very seldom applied. It was established, however, that serum applied in appropriate doses is retained in the organism of experimental animals for 7-10 days thus creating persistent immunity. In England, at the present time, there functions a number of centers where at any time of the day one can obtain serum for prophylaxis and treatment of malignant anthrax [2]. In Switzerland, anti-anthrax serum for prophylaxis is administered subcutaneously in a dose of 20 ml [8].

In view of the fact that the cutaneous form of malignant anthrax is treated successfully with antibiotics [penicillin, aureomycin], serotherapy is recommended only in serious forms of the disease, in conjunction with antibiotics. According to data of American authors serum in conjunction with antibiotics is administered to patients at a daily rate of 50-100 ml intramuscularly until an effect is obtained [9]. English authors recommend an initial intravenous serum introduction of 40 ml to anthrax patients, and subsequently, according to necessity, to repeat the administration of same several times per day. In serious cases serum is introduced at a daily rate of 300 ml for five days [3]. In France serum is applied in a complex with sulfanilamides and antibiotics or without them in a dose of 60 ml administered subcutaneously, with subsequent injections repeated daily.

until the clinical effect is produced [4]. According to data of Swiss authors, serotherapy is not effective in the cutaneous form of the disease. The serum is applied repeatedly either intravenously, intramuscularly, or subcutaneously at a dose of 20-50 ml until a clinical effect is produced. In serious cases, serum is introduced in doses of 100-200 ml in conjunction with antibiotics [8]. American authors emphasize that in anthrax, produced by a pathogen resistant to antibiotics, serum therapy will undoubtedly play a substantial role in the complex of medicinal measures [7]. Therapeutic serum dosages, adopted in some countries, are presented in table 8.

Table 8

		USA	England	France	Switzerland
Prophylactic dose		--	--	--	20 ml
Therapeutic Dose	Daily dose [first few days]	50-100 ml [with anti- biotics,	40-300 ml	60 ml	100-200 ml [with anti- biotics]
	Dose for the entire course of treatment	Until an effect is produced	Up to 1,500 ml	Until an ef- fect is produced	Until an effect is produced

**PURIFICATION AND CONCENTRATION OF SERA.** As is well known, animal serum [produced by horses] contains 6-9% protein in which albumins comprise 3-4%, euglobulins 1-2%, and pseudoglobulins 2.5-4%. It was established that in therapeutic antitoxic sera the main carriers of protective properties appear to be pseudoglobulins, which are thermostable globulins, containing up to 70-80% of the antitoxin.

Purification and concentration of antitoxic sera is based on the removal of inactive inert proteins such as: euglobulins, albumins, and the inactive portion of pseudoglobulins.

Abroad, at the present time, purification and concentration of antitoxic sera consists, on the whole, in the application of the enzymatic hydrolysis method, in conjunction with thermal coagulation, based on the previous splitting of serum's protein molecules with pepsin [or trypsin] with the subsequent isolation of the thermostable fraction of globulins associated with the antitoxins. As a result of the breakdown of protein molecules, horse sera purified in this manner

lose their specificity as protein antigens and are almost deprived of anaphylactic properties; in addition to that, a considerable concentration of the antitoxin per unit of serum volume is attained; this permits volume reduction of the administered preparation into the organism. In connection with this, it is necessary to mention that in the purification a certain portion of the serum's antitoxins is lost. The antitoxin losses, fluctuating from 20 to 50 per cent [17], depend on the purification method.

The enzymatic hydrolysis method, subject to various modifications is applied abroad.

In Denmark, at the State Institute of Sera [Copenhagen], the Hansen method is adopted [13, 14]. It involves the preliminary removal of fibrin and the antiproteolytic enzyme from the plasma with ensuing peptic digestion. A selective absorption on aluminum hydroxide gel, and removal of non-specific proteins is performed subsequently. After ultrafiltration through collodion covered candles, the specific globulins are salted out by the addition of ammonium sulfate followed by dialysis in cellophane bags.

The concentration coefficient depends to a large extent on the initial serum titers, the average value of which is equal to 5; the antitoxin yield comprises 70-80%.

In spite of the high yield, the method is very inefficient and not suitable for the processing of large serum volumes; [besides Denmark] it is not adopted in other countries [13, 14].

In France, at the Pasteur Institute [Paris] the Amoureux method [15] is applied. It involves the peptic digestion of the serum proteins followed by selective coagulation with ammonium sulfate at a temperature of 56.6-57°; the non-specific coagulated proteins are removed subsequently by filtration. The French method is characterized by the depigmentation of the serum with kaolin and carbon followed by filtration. The specific globulins are subsequently precipitated with ammonium sulfate and subjected to dialysis for a 30 hour period, after which a sterilizing filtration is performed.

For an anti-diphtherial serum, the yield of antitoxins comprises 50-55%, while the protein content at an initial 1,000 AE titer is 8-11%; for an anti-botulin serum, the yield is equal to 40-45%, while the protein content at an 1,500 AE titer comprises 5-8%. Electrophoretically, a single fraction is determined in the purified serum [14, 15].

In England, at the Wellcome Laboratory, the Harms method is applied [16]. It involves likewise the digestion of the serum proteins

in conjunction with thermal treatment at 55° and salting out with ammonium sulfate. The inert proteins, which are removed by filtration. The antitoxic globulins remaining in solution are thereupon salted out again with ammonium sulfate; they are then isolated and subjected to prolonged drying in a stream of flowing air. Dialysis in cellophane bags and sterilizing filtration are performed subsequently. The overall purification coefficient amounts to approximately 5.8, the antitoxic field comprises 65% and the maximum titer is 100,000 U. per ml.

Finally, in the USA, at the Michigan State Military Institute, a unique method is adopted. It begins with peptic digestion of casein, but, after which thermal denaturation of the proteins at 50° for 5 hours is performed; this is followed by the precipitation of inert globulins with ammonium sulfate and their subsequent removal. The next step involves the first precipitation and removal through filtration of antitoxic globulins remaining in solution.

The isolated antitoxic globulins are subjected to dialysis, conservation, and depigmentation (by alums), after which the second sedimentation of antitoxins with ammonium sulfate is undertaken. Following filtration, drying, sulfate removal (by a press), and dialysis, sterilizing filtration is performed (14).

Therapeutic sera abroad, particularly in England, are manufactured both in the liquid as well as in the dry form. Liquid native sera have a yellow or yellow-cinnamon coloration. Solutions of purified and concentrated sera are usually almost colorless, transparent, and odorless, with the exception of the preservative's odor, occasionally, however, they can have yellow-cinnamon or greenish-yellowish colorations. Dry preparations are white or yellowish-white powders. The pH of native sera varies from 7.0 to 8.5 (depending on the bicarbonate concentration of bicarbonates), the pH of purified and concentrated sera is equal to 6.0-7.0. Both native as well as purified sera can be stored at a temperature of 0° for five years; at a temperature of 5° for one year, the sera do not lose more than 5% of the preceding year's activity. At higher temperatures, the deterioration rate of the preparations rises: at 15° for one year, it attains 10%, at 25°--20%, while at 37° their loss of activity varies from 15 to 50%.

Sera purified by enzymatic hydrolysis are preserved better: at a temperature of 0-5°, the annual activity loss is very small; even at 15 and 20° it does not exceed 5 and 10%, respectively; at 37°, the preparations lose annually from 10 to 20% of their activity.

Dry preparations are preserved even better. Storage under absolutely dry conditions at a temperature below 10° results in practically no change in their activity; elevation of storage temperature



to 20° causes a very insignificant lowering of activity [3].

**GAMMA-GLOBULINS.** For the last 10-15 years gamma-globulin, prepared from human blood, has received widespread attention in the prophylaxis and treatment of certain virus and bacterial infections. Gamma-globulin represents a blood protein fraction which appears to be the main carrier of anti-virus and anti-bacterial immunological properties of therapeutic sera, and differs from other serum protein fractions by its electrophoretic characteristics [rate of migration in an electrical field].

However, the direct isolation of gamma-globulins by way of preparative electrophoresis can not be realized on a large scale. Therefore, in order to obtain gamma-globulin under manufacturing conditions, other methods, especially the fractionation method of serum proteins through a low temperature precipitation with alcohol are employed. This method is practised on the largest scale abroad.

At the present time, gamma-globulins are applied successfully for treatment, mainly for special skin prophylaxis, poliomyelitis, Botkin's disease, scarlet fever, epidemic parotitis, German measles, infectious mononucleosis, as well as smallpox. Considering that in many of the enumerated diseases, population is gotten over with in childhood, and that effective and systematic vaccination against smallpox is prevalent, it is assumed, that blood [serum] population possesses to a considerable extent protective properties against these infectious diseases. Departing from this point of view, for prophylaxis and treatment of the above enumerated infections, gamma-globulin prepared from normal serum of adults is applied. Moreover, each batch of the preparation is prepared from plasma or serum, derived from 500 people [3,5,10,11,12].

The main advantage of gamma-globulin, in comparison with serum appears to be the considerably higher concentration of protective antibodies per unit volume and removal from the preparation of the major portion of inert substances contained in the serum. Thanks to this, the prophylactic or therapeutic dose of antibodies is introduced into the organism in a considerably reduced volume (by 10 or more times), thus securing a lowering or complete absence of reactivity and anaphylactogenesis of the preparation [10].

The stated data is presented only as a general characterization of gamma-globulin, because pathogens of the skin, poliomyelitis, and Botkin's disease are not regarded by foreign specialists as possible bacteriological warfare agents. In this respect, foreign data concerning experiments with gamma-globulins for special prophylaxis of smallpox is of much greater interest. Initial observations

regarding the prophylactic effectiveness of gamma-globulin at the time of a smallpox outbreak were obtained in India. Later works by American authors [at the University of California] performed in 1953-1954 have established that gamma-globulin, derived from persons, vaccinated against smallpox, contained many virus-neutralizing antibodies. Subsequently, epidemiological observations were conducted. At the time of two smallpox outbreaks in India, all the persons who came in contact with patients were vaccinated in the usual manner against smallpox. Within 12-24 hours after vaccination, gamma-globulin, immune to virus of smallpox blood, in the amount of 0.06 ml per kg of weight was administered to some of them; the gamma globulin was obtained from blood donors who were vaccinated against smallpox 1-2 months prior to blood withdrawal from them and who displayed a positive reaction. Out of a total of 75 people who were only vaccinated in the normal manner, nine were taken ill with smallpox. Among 54 people to whom gamma-globulin was administered, there was not even a single case of disease contraction.

It is assumed that normal smallpox vaccination performed 2-3 days after infection, does not protect a person from getting sick. Gamma-globulin, containing antibodies against smallpox virus, has in this respect a considerable advantage [17].

In this way the problem of producing immunological gamma-globulins from human serum of persons suffering from certain diseases or who are vaccinated against them is solved at the present time.

However, in conjunction with this, the principal possibility of preparing immunological gamma-globulins from hyper-immunological therapeutic sera derived from animals was demonstrated during the last few years. Anti-plague gamma-globulins are especially so produced.

In this way, substantial development of a number of sera against virus and bacterial infections which at the present time can not be considered as sufficiently effective [plague, anthrax, seasonal encephalitis, and others] has emerged.

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## ANTIBIOTICS.

Antibiotics, as well as vaccines and therapeutic sera belong to a series of specific agents of individual protection against bacteriological weapons. Their main significance lies in the treatment of infectious diseases which can manifest themselves after a bacteriological attack. The presence of effective preparations with regard to a considerable number of infections, the agents of which can be employed in the arsenal of bacteriological weapons [plague, tularemia, malignant anthrax, rickettsia, psittacosis, and partly brucellosis] permit us to rely on the fact that specific therapy can reduce significantly the mortality rate among the sick as well as ease the course of the diseases and shorten their period of duration.

According to official views [9,10,11,12], antibiotics and chemopreparations under conditions of bacteriological warfare will find application not only for specific therapy of infectious diseases, but also for special prophylaxis. Underlying this fact is the creation in the already infected organism of a therapeutic concentration of the medicinal preparation which will prevent the disease's development or facilitate its course. It is assumed that antibiotics and chemopreparations will be employed for this purpose as soon as possible after application of bacteriological weapons.

Inclusion of special prophylaxis by antibiotics into the anti-bacteriological defense system has led American specialists to point out at the same time a series of shortcomings of this method. To begin with, it can not be applied for infections for which there are no effective antibiotics as well as for diseases produced by medicinally stable strains; secondly, execution of lengthy and repeated prophylactic courses produces sensitization towards the preparations and manifestation of side phenomena in a considerable number of persons subjected to prophylaxis. Finally, in the third place, in the opinion of USA specialists special prophylaxis by antibiotics can be begun only after ascertainment of the kind of bacteriological weapon employed, i. e. at a later time

when its effectiveness is reduced considerably.

The indicated circumstances narrow down sometimes the method's significance and permit one to recommend it for the prevention of diseases among confined troop contingents and the population [functioning military enterprises and so forth] [9,11].

It emerges from various foreign literature data that antibiotics and chemopreparations, employed for special mass prophylaxis, can be effective by causing a considerable morbidity reduction, by being capable of lowering the death rate and by facilitating the clinical course of the disease.

Abroad, especially in the USA, the most effective and widely applied antibiotics for the treatment of infectious diseases are penicillin, streptomycin, chloramphenicol, aureomycin, terramycin, and tetracycline. These antibiotics are of greatest importance for therapy and special prophylaxis of infectious diseases, the agent of which can be applied in the form of bacteriological weapons.

In the USA, moreover, during the last few years, antibiotics such as bacitracin, polymyxin, erythromycin, carbomycin, thyrothricin, nisin, neomycin, viomycin, furmagillin, rimocidin, etc., are developed in small amounts. However, they are employed mainly in surgical, therapeutic, urological and other diseases, and play a considerably smaller role in the treatment of infectious diseases.

**PENICILLIN.** The most studied and widely applied antibiotic in medicine is penicillin which, on the whole, is manufactured industrially in the USA in the form of benzylpenicillin and its preparations. Penicillin is of interest as a preparation against malignant anthrax and psittacosis and is indicated for special prophylactic purposes against gas gangrene and tetanus.

Although penicillin's chemical structure is well known, its synthetic manufacture is still not developed; the antibiotic is produced biosynthetically. In its dry form when stored under refrigeration, the preparation preserves its activity for one year.

In the USA penicillin is manufactured in the form of various preparations, intended mainly for parenteral [intramuscular, subcutaneous, intravenous] introduction, as well as for internal [tablets, suspensions], and external application [ointments, pastes, mixtures, powders, etc.].

Penicillin enters the blood stream within 30 minutes after intramuscular administration and is excreted from the organism mainly

through the kidneys. moreover, the therapeutic penicillin concentration is maintained in the blood for a total period of only 2-3 hours, which appears to be a **shortcoming of the preparation**. In connection with this, numerous preparations, so-called penicillin activity sustainers were created; in particular, significant successes in this area were attained by **deriving** various suspensions of penicillin procaine salt. For the last few years the preparation of bicillin or benzethacil representing the penicillin salt with N,N'-dibenzylethylenediamine deserves special attention. When 500,000 bicillin units are injected intramuscularly, the preparation is detectable in the blood stream for a period of one week; by raising the dose to 2,500,000 units, the penicillin can be detected in the blood after 15 days. Its concentrations in the blood are, however, relatively low, therefore bicillin is especially recommended for treatment of diseases caused by the most sensitive microbes towards penicillin.

An analogous preparation with regard to its prolonged action, under the name of benenic is being offered in England.

For treatment of pulmonary, pleuritic, and bronchial diseases, penicillin esters in the form of water suspensions, administered intramuscularly, are employed; these preparations ensure the creation of higher penicillin concentrations in the lungs and produce sustained activity.

In this way the problem regarding penicillin's sustained action is solved to a certain extent through the production of penicillin and bicillin procaine salts as well as through the creation of penicillin esters. However, in the opinion of American authors, in acute cases, intramuscular administration of penicillin per every 3-4 hours is necessary, a fact which indicates the relatively low quality of the sustaining preparations.

Penicillin when taken by mouth is rather quickly inactivated by gastric juice acidity. Therefore it is taken internally in the form of tablets, thus protecting the preparation from the acid's destructive action. In order to raise the therapeutic effect, administration of larger doses of the antibiotic and its application over a prolonged period of time is necessary. The acid-stable, biosynthetically produced penicillin phenoxymethylpenicillin, is intended for oral administration.

Complex preparations of penicillin with other antibiotics and therapeutic substances have found widespread propagation in the U.S.A. For instance, the following preparations were produced: penicillin + streptomycin; penicillin + quinine; penicillin + emetine; penicillin in conjunction with symptomatic agents, and many others [1,4].

According to data of American authors, for treatment of malignant anthrax's cutaneous form, daily intramuscular administrations of 200,000 to 500,000 penicillin units is recommended until temperature reduction and reversible development of local phenomena (usually within 5-7 days) is effected. In serious cases, the daily penicillin dose is raised to 4,000,000 units. In the treatment of malignant anthrax's cutaneous form, penicillin affords 100% recovery; in the opinion of American authors, therefore, there is no necessity in the application of serotherapy [6,10]. Treatment of anthrax meningitis with penicillin does not yield an effect in spite of applying large doses of the preparation.

In the treatment of psittacosis penicillin is applied in large doses. Intramuscular administration of 100,000 units per even, three hours during a minimum period of ten days is recommended. Complex therapy of penicillin with aureomycin, chloramphenicol or terramycin is not recommended [7].

Penicillin is an antibiotic devoid of significant toxic properties, and in connection with this, it can be applied in large doses for a prolonged period of time.

STREPTOMYCIN - ranks as the second antibiotic (after penicillin) according to the extent of production and investigation; it is manufactured industrially in the USA in the form of pure streptomycin and its dihydrostreptomycin derivative. The latter is a more stable preparation and, in the opinion of a number of authors, possesses lower neurotoxicity.

Streptomycin's chemical structure has been studied thoroughly. In the dry form at room temperature, the preparation can be preserved in its active state for a period of two years. The antibiotic is produced by fermentation. Streptomycin, of interest as an antibiotic is effective against the plague, and renders a high therapeutic effect in tularemia. It can be applied in the treatment of malignant anthrax in conjunction with therapeutic serum.

In view of the poor absorbability in the gastro-intestinal tract, streptomycin and dihydrostreptomycin are manufactured mainly as preparations for parenteral administration in the form of the following water soluble salts: the sulfate and hydrochloride as well as in the form of the calcium chloride complex which can be applied intralumbarily.

In parenteral (intramuscular, subcutaneous) introduction, both streptomycin and dihydrostreptomycin enter the blood stream; after a 24 hour period, 60-80% of the preparation is excreted by the kidneys.

Streptomycin is taken internally as tablets only in those cases in which local action of the antibiotic on the inciter, located in the gastro-intestinal tract, is necessary. Thus for the treatment of bacillary dysentery in children, the preparation of guaninyoin, manufactured in the form of tablets or water suspensions, representing a streptomycin and sulfaguanidine mixture is recommended.

In order to prevent the formation of streptomycin-resistant strains of bacteria, new salts and derivatives of the antibiotic are currently being produced; these demands are satisfied to a considerable extent by the streptomycin salt, streptomycin "Paskate", manufactured in the United States.

The recently produced streptomycin oleate is less toxic than the sulfates of streptomycin and dihydrostreptomycin, and is more slowly eliminated from the blood stream.

In addition to this, a number of complex antibiotic preparations are currently being manufactured: streptomycin + penicillin; dihydrostreptomycin + penicillin for parenteral administration; streptomycin + polymixin + bacitracin in the form of tablets.

In conclusion it is necessary to note that at the present time the assortment of streptomycin therapeutic forms is limited and is far from satisfying fully clinical demands [2,4].

American authors [Meier] recommend to carry out the treatment of the plague pulmonary and septic forms with a 3 gram daily dose of streptomycin in combination with aureomycin, chloramphenicol or terramycin, taken at a daily dose of 2-4 grams. There are indications that serotherapy supplementation to the above described plan yields positive results [6]. Welch [6] proposes treatment of the plague bubonic and pulmonary forms according to the following plan: a daily streptomycin dose of 2-4g, administered intramuscularly; sulfadiazine, given at an initial shock dose of 4g, followed by a 1-1.5g dose every 4 hours, taken internally (or intravenously). Length of treatment is 7-10 days; if improvement sets in during this time, streptomycin is discontinued, while treatment with sulfadiazine is continued [10].

Streptomycin is regarded as the most effective preparation in the treatment of tularemia. For therapy, 7-10g of the preparation is recommended; treatment is continued for 7 days, a daily 1-2g dose of the preparation being administered intramuscularly [10].

In the treatment of malignant anthrax's cutaneous form, streptomycin is applied intramuscularly at a rate of 3.5g given twice daily until a therapeutic effect is attained (disappearance of edema,



reduction of temperature to normal, and loss of toxicity symptoms). In serious cases, it is necessary to combine streptomycin therapy with serum treatment [at the rate of 50-100 ml per day]. In malignant anthrax, streptomycin should be applied only in the absence of an effect resulting from penicillin or aureomycin treatment.

Streptomycin is relatively non-toxic; however, during its application side reactions can be noted. Its toxic activity can manifest itself mainly through allergic reactions (skin rashes, pains in joints, fever, dermatitis) and function impairment of the hearing nerve (vestibular disorders, hearing impairment) [2].

Chloramphenicol (an analogue of Soviet levomycetin), is an antibiotic with a thoroughly investigated chemical structure, being manufactured synthetically. The preparation possesses a broad spectrum of activity and yields a therapeutic effect in the treatment of tularemia, typhoid fever and paratyphoid, acute bacterial dysentery, typhus, and other rickettsial diseases; it is also effective in the treatment of brucellosis and melioidosis. It is recommended in combination with streptomycin for treatment and special plague prophylaxis.

Chloramphenicol is well absorbed from the gastro-intestinal tract, ensuring a therapeutic concentration in the blood for a period of 4-6 hours. In connection with this, the antibiotic is employed for the most part internally. It is applied **perorally** in the form of capsules, tablets, and suspensions. It is used in the USA for treatment in children in the palmitate-ester form, containing 51.7% of the antibiotic. This preparation does not have a bitter taste and ensures a prolonged action. For parenteral introduction, chloramphenicol should be dissolved in N,N'-dimethylacetamide or dimethylformamide [4].

In the treatment of tularemia, chloramphenicol is applied internally at a daily dose of 2g for 5-6 days; for the entire course of treatment, 10-12g of the preparation are required [7].

Chloramphenicol appears to be a better therapeutic agent in the treatment of typhoid fever and paratyphoid. The initial daily dose consists of 0.25g per kg of patient's weight. Subsequent doses are administered at a rate of 0.75g per every six hours or 1g per every eight hours. Such treatment is continued until lowering of the temperature to the normal level is attained. This having been accomplished, the dose is reduced to 0.5g with an intake every six hours. The course of treatment with chloramphenicol is continued for 14 days [7].

Chloramphenicol is effective against bacterial dysentery, for the treatment of which American authors recommend a short course as follows: an initial shock dose of 2g, followed by four intakes at the

rate of 1g per every six hours.

For the treatment of rat typhus, chloramphenicol is applied in capsules at a dose of 0.5g per every six hours; the daily dosage comprises 2 grams (maximum dose is 6g). In the majority of cases the temperature falls within 72 hours. Treatment is stopped within 24 hours after the fall of temperature is effected [7,10].

Treatment with aureomycin, however, is more effective.

Chloramphenicol proved to be effective against rocky mountain spotted fever. The initial daily dose is administered internally; it is fixed at 0.05g per kg of patient's weight; the preparation is given subsequently after every eight or four hours at a dose of 1 and 0.5g, respectively. Therapy is continued until lowering of temperature and establishment of normal values within 24-48 hours is effected. When the patient is in a state of coma, chloramphenicol is introduced intravenously at a dose of 0.5g per every six hours; for this purpose chloromycetin hydrochloride is dissolved in a 5% glucose or a physiological solution [7,10].

Chloramphenicol is highly effective in tsutsugamushi fever. The initial shock dose is equal to 3g, after which the preparation is employed in a dose of 0.5g per every six hours. Normally the temperature falls during the first days of treatment. Therapy is continued for 24-72 hours. When treatment is started early (between the 4-6th day of the disease and sooner), in most of the patients relapses make their appearance; however, they are easily cured by a short repeated therapy course (3-5g), or they can be prevented through a single intake of chloramphenicol (3g) on the sixth day after completion of the first course of treatment [8].

The effectiveness of chloramphenicol in Q fever is lower as compared with aureomycin and terramycin. The preparation is applied, therefore, only in these cases when aureomycin or terramycin have not produced any desirable results during five days of treatment. Chloramphenicol is introduced in a dose of 2-4g per day until the clinical effect is attained followed by an additional three days after lowering of the temperature [3].

Reports are available concerning the fact that chloramphenicol is effective in the treatment of brucellosis, although it is less efficient than aureomycin itself or aureomycin in conjunction with streptomycin; the performed course of treatment does not guarantee the manifestation of setbacks. Treatment is continued for 2-3 weeks, a 2g daily dose of the preparation being given to the patients. In order to prevent the recurrence of relapses, it is necessary to repeat from time

to time the course of treatment [3].

According to scattered reports of French authors from Indochina, chloramphenicol gives an effect in the treatment of melioidosis.

The average daily dose comprises 5g, while that for the entire course of treatment amounts to 50-60g; the course's duration ranges from 10-20 days. Data regarding the successful joint treatment of melioidosis with chloramphenicol, aureomycin, and terramycin is available.

When large doses are administered, and during prolonged application, chloramphenicol can render a toxic effect on the organism. The toxic reaction resulting from an internal intake expresses itself mainly as nausea, vomiting, diarrhea, as well as in the outbreak of allergic rashes and changes in the mucous membranes of the oral cavity and sex organs. Blood changes (anemia, decrease in the number of corpuscles), deserve particular attention.

Aureomycin (an analogue of Soviet biomycin, is an antibiotic with a broad spectrum of activity, being effective against rickettsia, psittacosis, malignant anthrax, tularemia, typhoid fever and paratyphoid, being relatively effective in the treatment of brucellosis. It is recommended for the treatment of plague in conjunction with streptomycin.

Aureomycin's chemical structure has been well studied; however its synthetic production has not as yet been mastered; the antibiotic is manufactured by fermentation.

Aureomycin is well absorbed from the gastro-intestinal tract, ensuring a therapeutic concentration of the preparation in the blood for a prolonged period of time (about six hours). Therefore aureomycin for the most part, is used internally. In the USA it is manufactured in capsules (in doses of 50, 100, and 250 mg), and in the form of soluble tablets (in doses of 50 mg of the antibiotic), as well as in the form of preparations for intravenous and local applications.

The preparation for intravenous application represents a dry hydrochloride salt, buffered with sodium glycinate. The preparation is manufactured for local application in the form of ointments, tooth pastes, lozenges, solutions, and powders [3,4].

Aureomycin is considered as the most effective antibiotic against rickettsia. For the treatment of epidemic and rat typhus, a daily internal intake of 2g (maximum dose 6g, until clinical improvement and lowering of temperature to normal is recommended. Treatment is discontinued on the second day after establishment of normal temperature. When the patient is in a state of coma, the antibiotic is

applied intravenously at a dose of 0.5g per every eight hours as long as the patient is unable to swallow [7,16].

For treatment of Rocky Mountains spotty fever, a daily dose of 0.025-0.05g per 1 kg patient's weight, taken internally until attainment of a therapeutic effect, is prescribed. Treatment is stopped within 1-2 days after establishment of normal temperature. In persistent vomiting or in a state of coma, aureomycin is administered intravenously [7,16].

Aureomycin is highly effective against *tsutsugamushi* fever. The preparation is applied according to the plan adopted for chloramphenicol [8].

Aureomycin is recommended by American authors as the most effective therapeutic agent, together with terramycin, against a fever. The preparation is taken internally at a daily dose of 2-6g. The initial dose should be twice as large as any of the subsequent ones (shock dose). After reduction of temperature to normal, treatment is continued for an additional three days. When it is impossible to take aureomycin internally, the preparation is prescribed intravenously, the dose being 0.5g to be administered 2-3 times per day [7,16].

Aureomycin is the most effective preparation for the treatment of psittacosis. It is prescribed in a dose of 0.5g to be taken internally per every six hours for a period of ten days. For patients unable to take aureomycin internally, it is prescribed intravenously, the dose being 0.5g per every 12 hours [7,16].

Aureomycin serves as a good agent for treatment of malignant anthrax's cutaneous form. The preparation is applied internally, the dose being 0.75g per every four hours until clinical improvement is apparent.

In general the course of treatment is continued for about six days, the average dose for the entire course being around 20g [6].

Although aureomycin is less effective than streptomycin in the treatment of tularemia, it can also be applied in the treatment of this disease. Treatment is continued for a period of 5-6 days, the daily dose being 2g [7].

In the treatment of brucellosis, aureomycin is prescribed for a period of 2-3 weeks, the daily dose being 2g. Treatment does not guarantee the onset of the disease's relapses; in order to prevent setbacks, it is necessary to repeat the course of the aureomycin therapy [7].

Aureomycin is inferior to chloramphenicol as a means of treating typhoid fever and paratyphoid; however, it also yields positive results when identical doses of the antibiotic are employed [7].

Treatment of acute bacterial dysentery with aureomycin is performed according to the plan adopted for chloramphenicol and terramycin [5].

The preparation is relatively non-toxic, and the majority of patients endure treatment satisfactorily. However, in a number of cases, when the antibiotic is taken internally, the following toxic reactions caused by the gastro-intestinal tract can be observed: nausea, diarrhea, gastric upset, meteorism, manifestation of rashes and leucine-like lesions in the oral cavity. Many of the described reactions are so insignificant that they can be disregarded [5]. There are indications regarding the development of thrombocytopenia when aureomycin is employed intravenously.

Terramycin, as well as chloramphenicol and aureomycin, possesses a broad spectrum of activity, resembling in particular aureomycin according to its therapeutic effect [3].

Terramycin displays a good therapeutic action in the treatment of rickettsial diseases (especially in typhus) and gives a positive effect in the therapy of psittacosis, tularemia, salmonella enteritidis cutaneous form and bacterial dysentery, whereas American authors consider it as the most effective antibiotic for treatment of dysentery. Terramycin gives relatively positive results in the treatment of brucellosis and shortens the length of vibrio isolation in cholera; it can also be applied in conjunction with streptomycin for treatment of plague.

For treatment of the enumerated diseases, it is recommended to use the preparation in the same doses as those prescribed for aureomycin. Shortening of the vibrio isolation period in cholera is attained when a daily dose of 6-7.5g, administered for 3-4 days, is prescribed [6,7,8].

Terramycin as well as the majority of other antibiotics is manufactured by fermentation. It is well absorbed from the gastro-intestinal tract. It is produced abroad (in the U.S.A.) chiefly in the form of therapeutic preparations, designated for internal intake (capsules, as well as tablets, suspensions, drops, medicines). For intravenous introduction, terramycin is produced in the form of its hydrochloride salt, buffered with sodium glycinate which maintains the pH of an aqueous terramycin solution close to that of blood [4]. For intramuscular application, terramycin is manufactured in the form of its hydrochloride, magnesium chloride, and procaine hydrochloride [15].

Tetracycline (tetracyclin, tetracyclol) is an antimicrobial agent, according to its chemical structure and activity spectrum, related to very much aureomycin and terramycin. It is, however, less active than the last two compounds. During recent years, it has received widespread circulation in medical practice.

The preparation was received initially from aureomycin, 10 years of its crystalline hydrogenation. It was subsequently prepared from a cultural liquid of one of its strains, isolated from the cells of *Tetris*.

Tetracycline is manufactured in the form of capsules, tablets, and ampoules in capsules and tablets of 25, 100, and 250 mg. for internal use as well as in small bottles of 100-200 mg. for intravenous and intramuscular introduction. In intravenous administration, the preparation's maximum concentration in the blood is observed after one hour, while in internal intake [1] of tetracycline, after 4-6 hours. Its excretion from the organism is effected by renal excretion. Tetracycline's daily therapeutic dose corresponds approximately to doses of aureomycin and terramycin, being determined at a rate of 11-20 mg. per kg. of patient's weight; it is usually prescribed in 1-4 doses with interruptions of 3-5 hours.

Tetracycline finds application in the treatment of the same infectious diseases as aureomycin and terramycin, over which it has a number of advantages. Tetracycline is more stable in solution and is better soluble in water; when it is taken internally, a higher concentration is found in blood and spinal fluid; finally, tetracycline produces more rarely toxic manifestations.

Observations regarding typhus treatment with tetracycline are available. The prescribed daily dose is 400 mg. of tetracycline per kg. of patient's weight and is given in three doses; treatment is continued for a period of 3-4 days. Temperature reduction to normal was observed within 20-40 hours after start of therapy, and patients were discharged from the hospital on the tenth day. Complications and relapses were not noted, and toxic manifestations were observed rarely, even in treatment with aureomycin and terramycin [12, 13, 14].

Erythromycin (erythrocin) and carbomycin as well as tetracycline have received widespread circulation in therapeutic practice only during the last few years. The activity spectrum of these antibiotics resembles penicillin; for the most part, therefore, they find application in surgical, therapeutic, and other diseases, whereas in infectious diseases practice, they are of little significance. Both regarding erythromycin's and carbomycin's effectiveness against psittacosis deserves special attention [14].

Manufacture of antibiotics in the USA. Data regarding the antibiotics industry in the USA (as well as in England, Germany, and France), shows that production is carried out by the subversion method.

The continuous production method is much more economical than the cyclical one, and although the latter is being developed, it has not found until now widespread industrial application.

According to data of the American press [5], the current production rate of antibiotics in the USA exceeds demand. This applies for the first instance to penicillin and streptomycin.

In 1951, 558,000 Kg of antibiotics of all varieties were manufactured in the USA. This quantity comprised 234,000 kg of penicillin salts, 161,000 kg of streptomycin and its derivatives, and 140,000 kg of other antibiotics. In addition to that, 107,000 Kg of industrially manufactured antibiotics were designated for animal feeding and other agricultural needs.

During 1952 the industrial production of antibiotics increased to a certain extent. The penicillin and streptomycin productions amounted to 210,000 kg and 175 kg (350,000 and 175,000 billion units, respectively).

The production of three antibiotics with a broad spectrum of activity, chloramphenicol, aureomycin, and terramycin, comprised 24,000 kg in May of 1952, which, when calculated for the entire year, would yield 268,000 kg.

Overproduction of penicillin and streptomycin in the USA was caused to a large extent through reduction of their export. The amount of exported penicillin fell in 1950 from 31% [of the total output] to 21% in 1952; a similar situation existed also with regard to streptomycin.

A decrease in antibiotic exports from the USA was caused as a result of the development of antibiotic industry in other countries. Thus, France, for instance, has discontinued at the present time the import of antibiotics (in 1950, French plants manufactured 1.8 billion units of penicillin and about 1,400 kg of streptomycin). In Japan streptomycin production rose in 1952 to 2,140 kg (in 1950 it amounted to 116 kg) while that of penicillin rose to 10,300 billion units (in 1950, 7,500 billion units were produced). Available data indicates that there are at least 25 penicillin and 5 streptomycin plants currently operating in Japan. The output of antibiotics is considerable in England. Six concerns are engaged in the manufacture of penicillin there.

According to data of American authors, antibiotics and waste products of same industry are utilized successfully as animal feed. The annual cost of antibiotics utilized in agriculture of the USA comprises approximately 20 million dollars. Aureomycin, terramycin, bacitracin, and certain penicillin salts are utilized for these purposes. It is assumed that the quantity of antibiotics consumed as animal feed has to exceed the amount of preparations utilized for therapeutic purposes.

It should be mentioned in conclusion that the USA has a highly developed antibiotics industry, surpassing both in output and in production assortment the industrial capacity of any other capitalist country. The USA manufactures a variety of antibiotics which provide specific treatment for the majority of infectious diseases, the agents of which can be applied in the form of bacteriological weapons. Effective specific agents are available against the plague, tularaemia, malignant anthrax, typhoid fever and paratyphoid, bacterial dysentery, rickettsia, as well as psittacosis.

The capacity of the USA's antibiotics industry is very high. At the present time the USA's industrial output of antibiotics exceeds demand, the result being that a considerable portion of the production is being exported.

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## MEANS OF INDIVIDUAL AND COLLECTIVE NONSPECIFIC DEFENSE.

In the opinion of foreign military experts (1,2,3) the effect of bacteriological warfare agents has some common features with that of chemical poisons.

Bacteriological agents, as well as chemical poisons, can be disseminated in the air and shift with the winds (3).

According to the official view of the American Civil Defense Administration (4,5), one of the basic effects of a bacteriological attack is contamination of surface air by means of bacterial aerosols formed above towns and settled areas. As pointed out in the manual of the medical service of the U.S. Navy (3), the bacteriological agents can cause disease by penetration into respiratory organs, as well as infection of clothes, equipment, food products and water supply, which in turn become sources of contamination of people and animals.

Therefore, the main purpose of nonspecific defense against bacteriological as well as chemical and radiological warfare agents is the prevention of penetration of the agent into the human body and decontamination of the warfare agents on infected objects and areas (6). Therefore, the means of defense are also common to all the enumerated warfare agents.

According to American instructions and manuals (3,7,8,9), the means to prevent the penetration of microbes into the organism are similar to those used for defense against chemical warfare. They are divided into individual and collective defense measures. The means used for individual defense include gas masks, protective



Fig. 35 Latest American gas masks.

A. Light-duty gas mask

13. Field gas mask

clothes impermeable to biological agents, and also disinfectants for individual use; the collective means are shelters and dug-outs of various types and constructions intended for many people.

The principal means of individual defense is the gas mask. It protects the face, the eyes and the respiratory organs from penetration of aerosol particles. The anti-smoke filter located in the gas mask container keeps off the solid and liquid particles of the bacterial aerosol and purifies the inhaled air. The mask protects the face from warfare agents that hit the skin and the mucuous membrane. In the latest, Model M-9, American gas mask, used by the Armed Forces, a small mask container is attached to the front part of the mask near the cheek (see fig. 35). It is recommended to use cotton-gauze bandages and goggles if masks are unavailable or in bacteria-infected areas.

An important individual means for protection of the skin is a special protective apparel used for defense against particles of aerosol. As protective apparel, the American manuals (6,9) indicate overalls with an attached hood made of a special material which is impermeable to chemical vapors, radioactive particles and bacteriological agents (fig. 36). Rubber boots and gloves



Fig. 36. An impermeable protective garment

are part of the apparel. In addition to the impermeable overalls, American military experts (6) recommend impregnated outfits of a special cut which prevent the access to the body of radioactive particles and toxic vapors and impedes the penetration of particles of bacterial aerosol (fig. 37).

The impermeable outfit makes the evaporation of

perspiration difficult. The thermoregulatory disorders can lead to overheating of the organism. With the rise of temperature in the surrounding area, the overheating of persons dressed in impermeable outfits increases. Therefore, very strict time limits, depending on temperature, are prescribed for use of protective apparel.

According to American standards (6), the use of protective apparel at a temperature of  $32^{\circ}$  should not exceed 15 minutes, at  $29-32^{\circ}$  - no more than 30 minutes, at  $26-29^{\circ}$  - no more than one hour, at  $21-26^{\circ}$  - up to 1 hour, at  $10-15^{\circ}$  - up to 3 hours, at  $1-10^{\circ}$  - up to 5 hours; under  $1^{\circ}$  protective apparel can be used up to 5 hours.



Fig. 3/ Impregnated garment with a hood

To increase the duration of wearing the protective garment, the American army uses a so-called cooling dress on top of the overalls.

This dress (M-1) is composed of a jacket, pajama-like trousers, and a hood made of "swaddling" cloth. The trousers are fitted out with suspenders and clasps for adjustment of the cuffs. The sleeves and the back of the jacket as well as the hood are made of two layers to

increase the cooling effect.

The cooling garment is periodically moistened with water. The evaporation of water cools the protective garment and permits its longer use in warm weather (6).

Various kinds of shelters and dug-outs are used as collective means of defense against bacteriological, chemical and radiological warfare agents. As mentioned before, aerosols can penetrate with the air currents into non-hermetic installations, buildings, cracks, mud-huts, etc. (10,11) and infect unprotected people. It is therefore required that shelters from chemical, radiological and bacteriological warfare agents should be completely airtight.

Taking into account the high destructive power of contemporary weapons, foreign experts attach great significance to protection of shelters from impact as well as from a direct hit. Building shelters against modern weapons does not present new problems, since many countries have had considerable experience in this matter. Therefore, this section will present only a short resumé of the material contained in recent American instructions and manuals (3,4,6,7,8,9,12).

It is customary to divide shelters into field shelters and permanent shelters, both of which can be of the ventilated and the non-ventilated kinds. Any available installations and material at hand are used in constructing the field shelters, while in building of permanent shelters provision must be made for a reliable protection against all types of weapons. It is especially recommended to build permanent shelters near important installations. According to American instructions and manuals, such installation in a military base are lines of communication, command posts, hospitals, etc., i.e. installations on which the uninterrupted functioning of the base during military operation depends.

In building and equipment of shelters provision must be made to enable the people inside the shelter to carry out all functions. In order to ensure such proper functioning, it is recommended to have underground shelters built of ferroconcrete. The shelters must be equipped with filter-ventilating installations and provide facilities for prolonged stays.

The American military experts attach great importance to the choice of proper sites for building of shelters. Consideration is given to a large variety of possibilities that can be instrumental in providing greater security to people in the shelter. Thus it is recommended, in choosing sites for shelters, to take into consideration the direction of winds and the topography of the area. A shelter built on the lee side of a slope is considered to be less affected by bacterial aerosol than one built facing the wind.

Building of shelters is not recommended in places where aerosol clouds are likely to remain at a standstill. In choosing a site, consideration should be given to the possibility of building horizontal or ascending entrances to the shelter. This will prevent penetration of aerosol and provide good drainage. The nature of the soil must also be taken into consideration. Most suitable is hard soil with good drainage. It is not recommended to build shelters in low-lying or swampy areas which will facilitate the penetration of bacteriological agents through soaking or by means of mud stuck to shoes or clothes.

If existing buildings should be used for shelters, it is essential to choose solid constructions, for example buildings with ferroconcrete frames, underground pillboxes or similar explosion-proof installations.

Special attention must be given to the construction of entrances to the shelter. Impacts and splinters from explosions can dislocate or destroy doors and lobbies and open the way for penetration of bacterial or chemical aerosols into the shelter.

To prevent this, the American experts recommend either to have entrances with a deflection angle of  $90^{\circ}$  or to build in front of the entrances sturdy partitions with side passages as impact-dampers.

Building of lobbies for shelters is obligatory. Their purpose is to hinder a possible penetration of aerosol into the shelter on entering or leaving it and to avert the collapse of the door brace into the shelter on opening the door. The lobbies must have metal doors or hermetic curtains made of impermeable material. The doors are to be lined with rubber so as to make them airtight.

The shelters may have several connected lobbies.

Specially constructed shelters, or underground constructions converted into shelters, are kept airtight by means of hermetic doors only.

The planning and equipment of shelters depends on their size and the purposes to be served. But, according to regulations, every shelter must be planned to provide satisfactory conditions. It must be equipped with sitting and sleeping facilities as well as with a medical unit. If water pipes or sewerage are not available, closed containers should be used for disposal of eliminations. An isolated area must be provided for people having or suspected of having contagious diseases. According to American regulations every shelter, irrespective of its construction, should have adequate space and be equipped with a disinfection room, an entrance connected with one or two lobbies, and an emergency exit.

It is required to have at least one emergency exit, and if possible more than one, in the event the main entrance is blocked by debris. The emergency exits should be at a distance from one another and easy to open from the inside.

Each shelter must have a reserve of water even if it has water installation. It must be provided with emergency food reserves, medicaments and a certain quantity of degassing and disinfecting remedies. It is advisable to have a supply of entrenching tools. If electric current is available, the shelter should be provided with electric light. Emergency storage-battery installation should also be available. It is not recommended to burn oil lamps or candles in non-ventilated shelters because of oxygen consumption.

It is also recommended to have a telephone installed in the shelters for communications with the outside world.

As a rule, ventilated shelters are equipped with filter-ventilating installations to purify the incoming air from chemical and biological agents. Such an installation, especially if powered by a gasoline engine, is placed in a separate room. If there is no such room, it should be installed outside of the shelter. In that



event, the installation should be protected from bombs, splinters and percussion action.

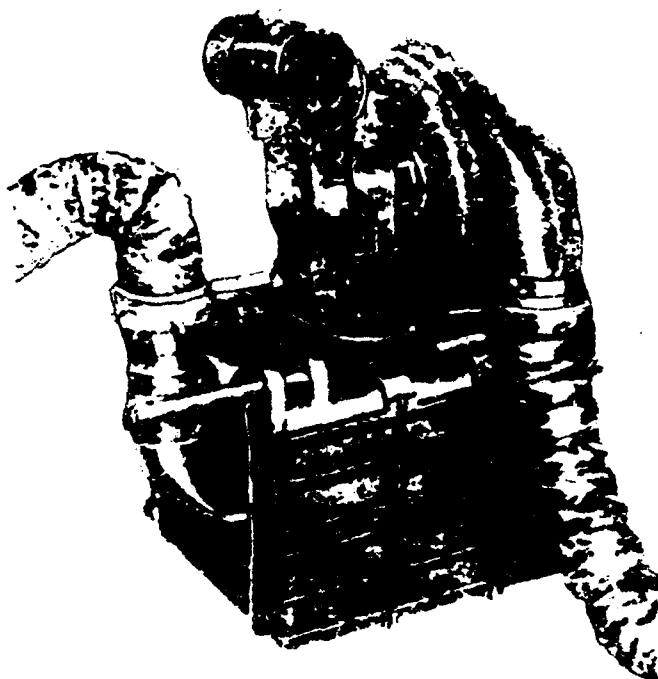


Fig. 38 Filter-ventilating installation  
M-6

The capacity of the filter-ventilator depends on the size of the shelter and the escape of air. The rate of incoming air should be in excess of the escape and thus create additional pressure, equal to about 2.5 mm. of the water column. This additional pressure will produce outward escape and thereby preclude a possible danger of penetration of contaminated air.

At present, the most powerful filter-ventilating

system used by the U. S. Armed Forces is army installation M-6 producing  $8.5 \text{ m}^3$  air per minute. The installation is composed of suction and filtration systems (Fig. 38). It has the following dimensions: 86.4 cm. long, 61 cm. wide and 99 cm. in height. Total shipping weight 304 kg. The filter-ventilation installation contains a filter-absorber, a fan, a motor, and air inlet and outlet bases. The filter-absorber is composed of seven detachable parallel sections: three air-distributing sections, two anti-smoke filters, and two charging sections. The sections are lined with neoprene rubber.

The pliable, rubberized outlet base is equipped at the end with a net to prevent penetration of debris, rodents, etc. The hose is 6 m. long and 12.7 cm. in diameter.

A shelter planned for extended stays is equipped with one or more filter-ventilating installations, depending on air-consumption.

American engineers have developed filter-ventilating equipment of a capacity range of 17,34,70 and  $140 \text{ m}^3$  per minute.

If properly constructed and operated, ventilated shelters provide a dependable protection from biological warfare agents.

In large permanent shelters it is recommended to set up sanitation wards for persons affected by aerosol clouds. These should be regular wards located at the entrance to the shelter. The scheme in Fig. 39 shows a practical layout for a sanitation ward in a shelter.

The holding capacity of shelters, especially of the non-ventilated type, must be strictly controlled.

The number of persons that can be admitted to the shelter for a given period of time depends on the volume of air in the shelter.

Experience has shown that in a relaxed condition a person consumes  $0.085 \text{ m}^3$  air per minute. It is recommended to use these figures and to take into account the amount of warmth given off by the human body, in determining the holding capacity of shelters.

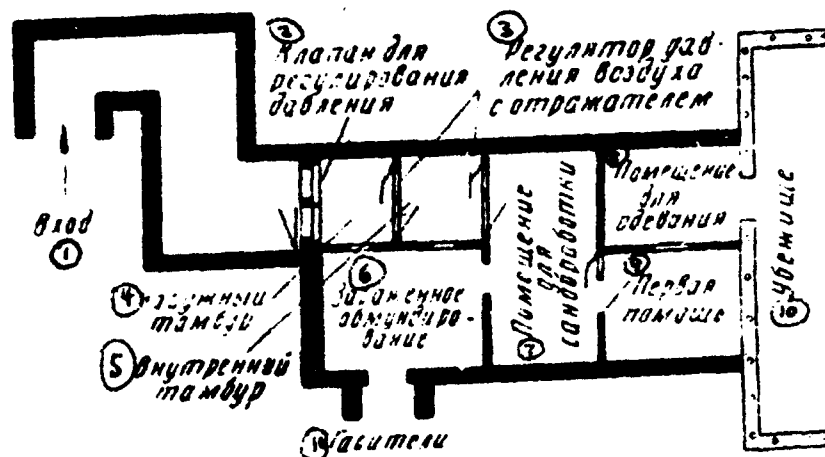


Fig. 39. Arrangement of Sanitation Area for the Shelter

- 1) entrance; 2) pressure regulation vent; 3) air pressure regulator with baffle; 4) outside vestibule; 5) inside vestibule; 6) contaminated clothing; 7) sanitary treatment area; 8) dressing area; 9) first aid; 10) shelter; 11) extinguishers.

Among collective measures of defense, mention must be made of degassing units for disinfection of persons caught in a cloud of a chemical, radioactive or bacterial aerosol. There are field and stationary degassing units.

It is recommended to set up a field degassing unit near a water source at a safe distance from other field institutions. The degassing unit is constructed and equipped similar to an ordinary sanitation ward. It contains a platform for disinfection of clothes and personal effects, a shower and a special dressing area (tents, etc.) for distribution of fresh clothes.

Degassing units of the stationary type are placed either in buildings or installations used for shelters, or in special constructions erected for this purpose. These locations, equipped with filter-ventilating installations, can be used as shelters against every kind of modern weapon (6).

## SEVERAL MODERN MEANS AND METHODS OF DISINFECTION

This section, compiled from American manuals and instructions published abroad in recent years, contains information of the latest achievements and experiences in the field of disinfection. Special attention will be devoted here to the description of new means and methods recommended by American disinfection experts for the elimination of after-effects of a bacteriological attack.

As pointed out earlier, bacteriological attacks may cause contamination of vast areas, including all the objects contained therein. The modern microbiological science, however, has not yet developed any methods or instruments for a quick determination of the nature of the biological warfare agents. It is even more difficult, if at all possible, to define the confines of the contaminated area. Considering the great expenditure involved in a large-scale disinfection operation, it is recommended in the American instructions (6) that a thorough evaluation be made of the situation in order to determine whether it is practical to carry out the operation. According to American sources of information, it is not yet possible to establish standards for definition of cases requiring disinfection. It all depends on the degree of importance of the contaminated objects for the war effort and the degree of resistance of the micro-organism used in the attack, since on discovery of the biological attack, which is usually made on appearance of the first symptoms in humans (or animals), a "natural" disinfection may take place and make additional interventions unnecessary.

Taking into consideration the difficulties in establishing the presence of contamination and determining its duration, the American experts recommend the evaluation of the need for disinfection in each particular case on the basis of its importance to the war effort. The most practical is the method that will reduce contamination to the point that it presents no danger, at a minimum expense of energy and materials, and in the shortest possible time. (6)

When a suspicion arises that the enemy is applying bacteriological warfare, it is recommended first of all to disinfect the objects with which the people have

direct contact. Under actual combat conditions this applies to weapons, combat outfits, as well as military equipment and important installations.

Disinfection should be applied to an area of tactical importance, while the rest of the affected territory should be left to "natural" decontamination. Entrance to this territory must be restricted and signs posted to this effect (7).

#### DISINFECTION MEANS

Since chemical and bacteriological warfare agents have common characteristics, it was possible for foreign experts to develop identical disinfection methods applicable to both. For example, the standard remedies developed by the Army for degassing of chemical contaminants are also used as disinfectants (6). Once common means and methods were found for disinfection and degassing, the two techniques were combined into one decontamination operation. In the opinion of American experts (8) who consider this combination of chemical and bacteriological methods practical, the application of one antidote to contaminated objects will be effective against both warfare agents. Needless to say that degassifiers are not recommended as a final solution to the entire problem of decontamination, because both chemical and bacteriological agents have many specific characteristics and therefore require specific methods of decontamination.

Let us now turn to the individual disinfectants which are the standard means used by the American Army (6).

Substance STB - settled calcium hypochloride mixture, or bleaching powder, containing 30% of active chlorine. It is distinguished from ordinary bleaching powder by a high content of unslaked lime (up to 13%) and a low rate of moisture. It can be used both for degassification and for crude disinfection, in liquid suspension form, or in a dry form mixed with neutral substances, (such as soil, sand, etc.) at the rate of 2:3 by volume.

It is recommended to use in degassing installations suspension matter at the rate of 40 parts STB to 60 parts of water by weight. In order to reduce the hardening

and increase the fluidity of the suspension matter, citric acid or sugar are added at the rate of 0,5 kg. to 100 kg. of STB. Because of the high cost of STB, degassification and disinfection by this method should be restricted to relatively small areas.

The approximate consumption of lime for degassification or disinfection is about 0.5 kg. per 1 m<sup>2</sup> of space.

In order to conceal the color of lime, ordinary lamp black at the rate of 1 kg. per 100 kg. of lime is added to the suspension.

For milder disinfections, chloramine T and dichloramine T can be used.

It is also recommended to use for disinfection the degassing solution DANC (decontaminating agent non-corrosive), which is a solution of the solid organic substance H-195, one of the chloramine compounds in tetrachloroethane. Substance H-195 is a white or cream-colored powder, of chlorine odor, which gradually decomposes when stored. Among its shortcomings are toxicity, a softening effect on rubber and plastic materials and the generation of hydrochloric acid on contact with humidity. It softens and destroys color, resulting in discoloration of painted surfaces and formation of spots. It has a mild corroding effect on metals. In view of these effects it is recommended to use it with caution and to wash with water the contaminated areas after application of this substance. Solution DANC is fluctuating when stored and should therefore be prepared in accordance with the quantities needed. The vapors of tetrachloroethane are poisonous and therefore gloves and gasmasks should be worn when using DANC.

In addition to the abovementioned substances, American instructions list a number of widely known disinfectants, such as: phenol, cresol, potassium permanganate, also the lately popular iodoforms, which contain iodine and substances that lower surface tension, thereby increasing the disinfectant effect. These substances are mainly used as liquid disinfectants.

Gas disinfectants become increasingly popular abroad, primarily ethylene oxide and mixtures of

ethylene oxide with other gas compounds.

Ethylene oxide is a slightly odorous liquid. Its specific gravity is 0.9 at 0° centigrade and its boiling point is +10.7° centigrade. It dissolves easily in water and in organic solvents. Ethylene oxide is widely used in the chemical industry because of adequate supply of this substance and its considerable chemical activity. As mentioned, it has a bactericidal effect especially on vegetative forms of microorganisms.

Among the shortcomings of ethylene oxide as a disinfectant are its relatively high toxicity and its explosive capacity when mixed with air.

To safeguard it from explosion, a mixture of ethylene oxide with carbon dioxide, the so-called carboxide, is used. Carboxide contains 10% of ethylene oxide and 90% of carbon dioxide and has the same effect as ethylene oxide. Carboxide is an effective disinfectant and is used in decontamination of enclosed areas, military equipment and ammunition. Carboxide is a standard part of equipment in the American Army (3,6). A similar disinfectant is Freoxide, which is a mixture of ethylene oxide with freon. All gas disinfectants are supplied in cylinders of various capacities and also in containers.

In addition to the enumerated disinfectants, American civil and military health organizations also use formalin and methyl bromide for disinfection.

In surveying the means of disinfection used abroad, mention should be made of various detergents which, even though not assuring protection against biological warfare agents, contribute considerably to disinfecting processes. The detergents remove the chemical and biological toxic agents from the soiled and greasy surfaces and act to a certain degree as dissolvents of organic compounds; the toxic agents are removed together with the organic compounds.

The U. S. Air Force uses the so-called emulsifying detergent MYL-C-25179, also called GUNK, for treatment of aircraft and other types of combat equipment. This substance is a mixture of alcohol, turpentine, soap, naphta and sulfurized castor oil. This detergent is

mixed with kerosene (1:9). The mixture is spread over the contaminated area by means of sprayers and after a while is washed off with a strong water stream out of a hose. The effect of water on the detergent produces an emulsion which washes off the biological and chemical toxic agents together with the dirt and grease. The emulsifying detergent GUNK is a standard degassifier in the military equipment of the United States Air Force. The U.S. Air Force uses in a similar manner another detergent, PS-751, which is also effective in degassing of chemical toxic agents which have a neuromuscular effect. This detergent is a mixture of phosphates, silicates, caustic soda, potassium and soap. Unlike GUNK, the PS-751 compound is used in a 5-10% water suspension or with steam. The suspended matter applied to the contaminated surface is left for 30 minutes and is thoroughly washed off with water stream to prevent corrosion of metal parts. When properly used, the detergents can remove as much as 90% of infectious matter from contaminated surfaces (6).

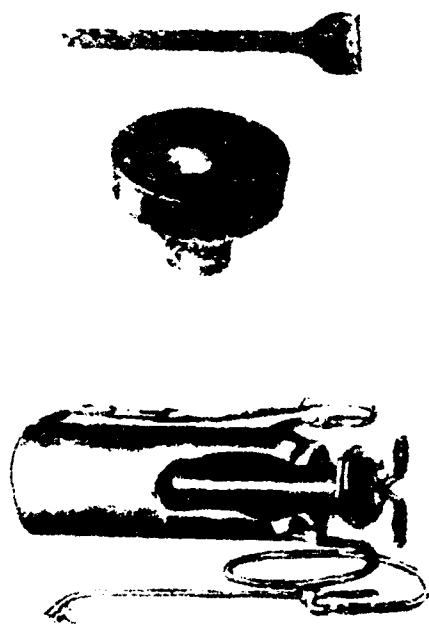


Fig. 40 Decontaminating equipment M-1

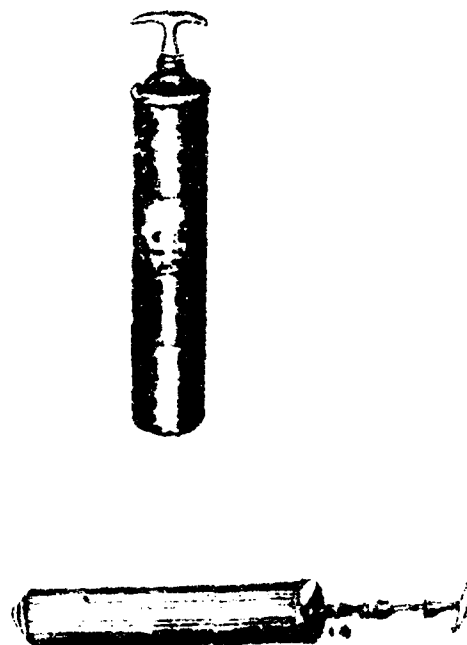


Fig. 41 Decontaminating equipment M-2



## DISINFECTING TECHNIQUE

The U. S. Army uses for disinfection portable and conveyable equipment which can also be used for degassification (6). The construction of portable degassifying equipment M-1 and M-2 (fig. 40 and 41) is based on the principle of automatic flow (M-1) and manual use (M-2) and they are used for the treatment of combat equipment and liquid disinfection of buildings and of limited open areas (M-1) (1,2,10)

The capacity of M-1 is 11.4 liters, that of M-2 - 1.4 liters.

An area of about 40 m<sup>2</sup> can be treated with one load of M-1.

For large-scale degassification (disinfection) the U.S. Army uses special degassing machines. They are used for treatment of localities, roads, airfields, combat equipment, as well as for the exteriors of buildings infected with chemical or biological warfare agents. One specimen of an American conveyable degassing equipment is the MZA machine (6). It is a steel tank of 1500 liters capacity mounted on the chassis of a truck. The tank contains rotary mixers for mixing of disinfecting solutions (suspended matter). The machine is also equipped with a three-cylinder piston pump for pressure sprinkling and with sprayers at the front. The pump and the mixers are set in motion by the truck engine.

The emptying time of the tank is 20 minutes at a working pressure of 28 kg/cm<sup>2</sup>. The main disinfectant used is a suspension of calcium hypochlorite. Eight hundred and fifty liters of water and 590 kg. of calcium hypochlorate are needed to fill up the tank (Fig. 42).

It is pointed out in the manuals that, in addition to degassification, these machines can also be used for water carrying, fire extinguishing and as field sprinklers.

A modified type of a degassing machine is a trailer spraying machine MV-1, which is a standard technical equipment of the U.S. Air Force (6). The equipment is composed of a tank of 1900 liters capacity, a pump, and a gasoline engine, all mounted on a trailer. The water-

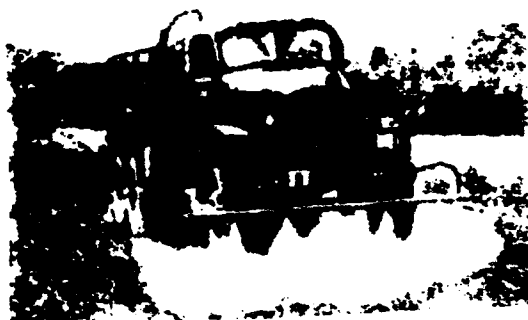
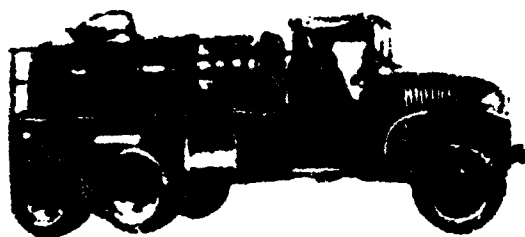


Fig. 42. Degassing machine MZA

ing system is equipped with front and rear sprayers with a discharge capacity of 19 to 132 liters per minute. A powerful high pressure pump supplies water at the rate of 75 liters per minute. It is also equipped with two hoses of 30 m. each (fig.43).

The purpose of this equipment is identical with that of MZA. It can be used for treatment of objects 17 m. high from a distance of 6 m. It can also be used for sprinkling of infected areas with so-called suppressing substances.\*

A portable water heater M-1 is used for hot water supply to the degassing machines, installations and other degassing equipment. It is a continuous-motion apparatus with a capacity for warming up 2300 liters of water to

\* Suppressing substances are water and oil products used for sprinkling of infected surfaces in order to prevent a secondary formation of aerosols. This is achieved by sprinkling of the soil or by creating of an organic coating over the infected area.

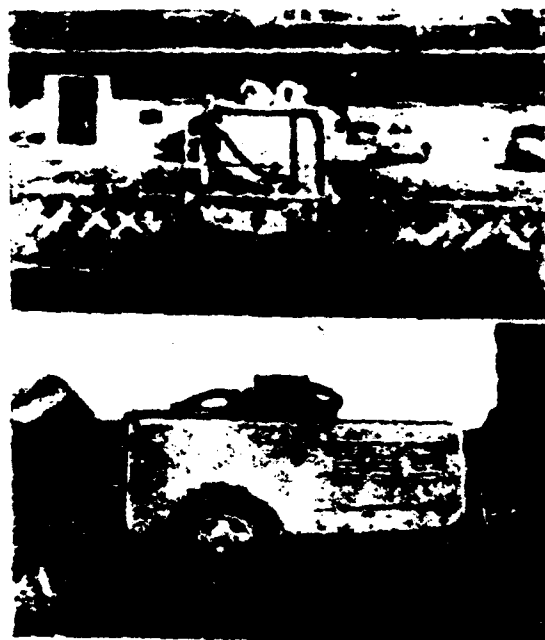


Fig. 43. Trailer spraying machine MV-1.

40° within one hour. The water heater contains a steel fuel tank, a liquid-fuel burner, a fuel pump serving also the heating element which is composed of two coils and a water tank.

The water is supplied to the heater by an intake hose from an elevated tank or by a pump. In addition to water heaters, the U. S. Army also uses steam producing apparatus. Their working principle is very simple: water is pumped into a heated coil and evaporates. The apparatus is equipped with a tank for the detergent (PS-751). The ejected steam is mixed with the dissolved detergent and is applied to the affected area.

For treatment of elevated objects, the U.S. Army uses a so-called elevator car MV-3. The elevator has the form of a platform which is mounted on a chassis of a truck. It can be lifted up to 15 m. A 1900-liter capacity tank for disinfectants with a hose and sprayer

are also mounted on the truck. The entire equipment is operated hydromechanically with an independent motor. It is used for treatment of airplanes. The danger of infection to persons operating this equipment is reduced to a minimum, since the treatment is done from above and the detergent is sprayed downwards, so that the sprinklers and the washed off liquid cannot hit the operators. Needless to say that persons working on any kind of degassing or disinfecting equipment must use protective apparel and gas masks.

#### METHODS OF DISINFECTION

According to American instructions on defense (6,9), army personnel caught in a bacterial aerosol cloud must be treated without any delay. They must take a hot shower with soap. In the field, treatment is given in showers of various types and constructions; under stationary conditions, however, this is done in sanitary wards and in bath houses.

Before undressing, the soldier's outfit is moistened with water (to prevent a secondary aerosol), and is disinfected by any available means (3,8) followed by mechanical laundering.

Woollen clothes and leather shoes are disinfected with methyl bromide or freoxide steam in special vapour-proof bags similar to those used for extermination of insects. In view of the toxicity of the fumigants and to avoid contamination, it is not recommended to carry out the disinfection in enclosed areas. Two sets of clothes can be processed in one bag. Five ampules of methyl bromide or a 0.36 litre container-dispergator of freoxide are put into the bag. The bag is hermetically sealed and the ampules are crushed. If freoxide is used, the helical valve of the container is opened. Then the bags are rolled on the ground for 5 minutes for a thorough penetration of the fumigants into the clothes. After 12 hours of fumigation, the clothes and the shoes are thoroughly aired out until the smell of the fumigant completely disappears.

The same treatment is recommended for articles of personal use (gas masks, mess-kits, eating utensils, leather and rubber articles, as well as precision

appliances and instruments).

The manuals indicate a variety of ways for disinfection of combat equipment. The mildest is the gas disinfecting method which eliminates corrosion of metal surfaces. Its shortcoming is the need for a prolonged period of exposure and it is therefore recommended to use liquid disinfectants whenever possible. In gas disinfection of combat equipment ethylene oxide is most frequently used. The equipment (guns, tanks, motor vehicles, etc.) is covered with a gas-proof tarpaulin with edges covered up with earth to prevent the escape of gas. A hose is placed under the tarpaulin and lets in the ethylene oxide which is fed from a cylinder. An

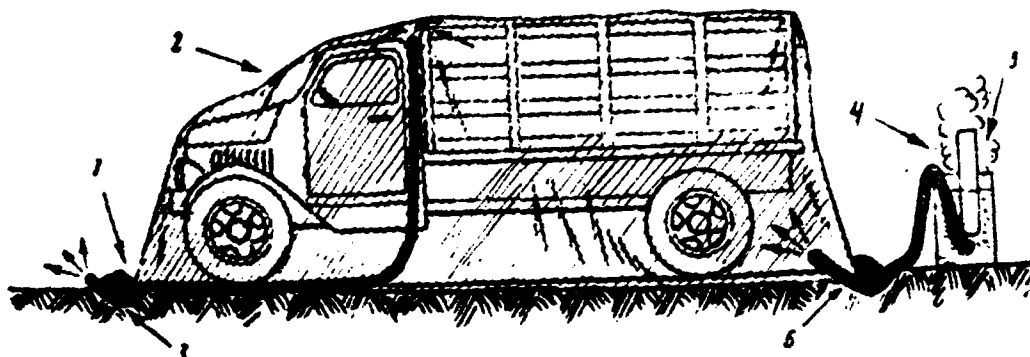


Fig. 44. Disinfection of a loaded truck with ethylene oxide.

- 1 - Edge of tarpaulin covered up with earth; 2 - gas-proof tarpaulin;
- 3 - outlet hose; 4 - barrel containing hot water; 5 - cylinder containing ethylene oxide; 6 - inlet hose.

air outlet hose emerges from under the tarpaulin. The ethylene oxide fills up all the space under the tarpaulin and pushes out the air through the outlet hose. As soon

are also mounted on the truck. The entire equipment is operated hydromechanically with an independent motor. It is used for treatment of airplanes. The danger of infection to persons operating this equipment is reduced to a minimum, since the treatment is done from above and the detergent is sprayed downwards, so that the sprinklers and the washed off liquid cannot hit the operators. Needless to say that persons working on any kind of degassing or disinfecting equipment must use protective apparel and gas masks.

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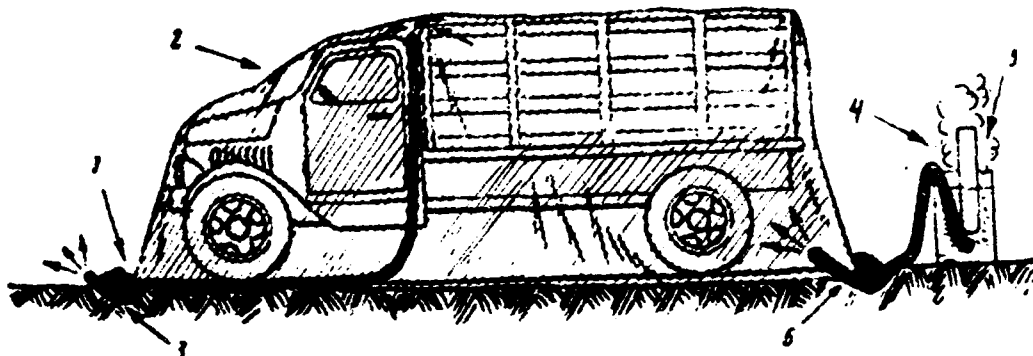


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- 3 - outlet hose; 4 - barrel containing hot water; 5 - cylinder containing ethylene oxide; 6 - inlet hose.

air outlet hose emerges from under the tarpaulin. The ethylene oxide fills up all the space under the tarpaulin and pushes out the air through the outlet hose. As soon

as the smell of gas appears at the outlet hose, the outlet is closed with a stopper while the inlet of gas is interrupted. The object is left under the tarpaulin for 24 hours. In order to establish the gas consumption, the cylinders are weighed. Ethylene oxide is poisonous and explosive, and it is therefore recommended to wear gas masks and keep the gas clear of fire and sparks.



Fig. 45. Disinfection of aircraft interiors.

Ethylene oxide can be applied to tents and buildings. The consumption of gas in treatment of a space of  $56 \text{ m}^3$  at a temperature of  $21^\circ$  and above is 27 kg. If the temperature drops to  $10^\circ$ , the quantity of gas should be doubled. The disinfecting action of ethylene oxide slows down at high humidity.

Since ethylene oxide is a very effective decontaminator and has no corrosive effect on metal surfaces, it is recommended to use it for machinery, precision appliances, instruments, ammunition, uniforms, etc. treated in a gas-proof chamber at a minimum exposure of 8 hours.

Disinfection with carboxide is treated in a similar



manner but twice the quantity of ethylene oxide is used.

One of the qualities of these fumigants is the high degree of their penetrability. It is therefore advisable to use them in treatment of tightly packed materials. If used for room disinfection, the room should be filled to excess so that the fumigant is fully utilized.

The high penetrability of ethylene oxide and carboxide, combined with their bactericidal properties, qualifies them for treatment of the interiors of ships, steamers, and defense installations, which are subjected to hermetic sealing (fig. 45).

In treatment of buildings where condensation of gas is likely, it is recommended to use formaldehyde, which is one of the best gas disinfectants. The application of formaldehyde is quite simple since it is not required to seal the building hermetically. However, this gas does not penetrate into tightly packed materials.

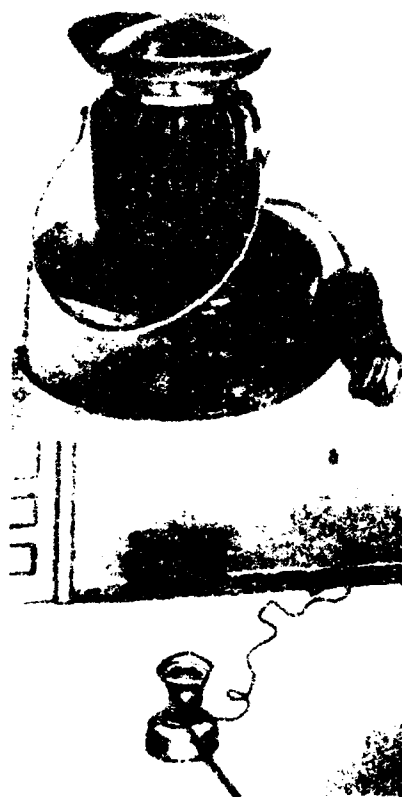


Fig. 46. Tockheim sprayer.



Fig. 47. Disinfection with steam.

Todt and Tockheim sprayers are used for dispersion of formaldehyde (fig. 46). The Todt sprayer can discharge 15 liters of formaldehyde in 8 - 10 minutes (6). The sprayer is mounted on a truck and the formaldehyde is dispersed with a hose directly from the truck. Barrels of water and formalin can be loaded on the truck and in this manner many buildings can be treated in a short time.

In treatment of outside surfaces of walls and buildings, of auxilliary machines, cars, ammunition and of similar objects, which to a certain degree are corrosion-resistant, it is recommended to apply liquid disinfection and calcium hypochlorite mixture, solution of DANC, phenol, cresol and other disinfectants, also hot water and steam. After treatment, the disinfectants should be washed off with soap and water, while metal surfaces should be greased in order to prevent corrosion.

#### Area Decontamination.

The treatment of open areas requires large quantities of disinfectants and considerable manpower. On the other hand, as it was pointed out earlier, due to difficulties in discovering a biological attack, a "natural" disinfection by meteorological factors may take place, making additional interventions unnecessary. Hence, the American manuals recommend first of all to take advantage of natural disinfectants and not to apply any additional treatment. Special signposts should be put up to indicate that the area is infected, and entrance into the area should be restricted or temporarily suspended. However, if admittance to the area cannot be stopped or limited, and if the sun radiation or the meteorological factors do not disinfect the area in time, it is recommended to treat with disinfectants such parts of the area as are operatively essential, e.g., roads, airstrips and airfields, military storage areas and other essential objects. It is recommended to use for disinfection the least expensive and the most readily available means, such as washing off the biological agents, preferably with hot water, and burning or steaming the infected area (3,6,9). If necessary, chlorine containing preparations (calcium hypochlorite mixture) and substances neutralizing secondary aerosols, should be used. Any moistening substance can be used as a neutralizer, provided the moisture remains on the surface for a considerable time.

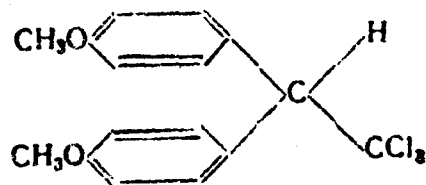
In hot areas, therefore, frequent sprinkling is recommended. If water is used, the moisture will last longer by adding a soluble oil to the water.

However, of all neutralizing substances, preference should be given to petroleum products (kerosene, mazut, diesel oil, waste oil, etc.). For spraying of areas with neutralizing substances, as well as with chlorine-containing solutions and suspended matter, use can be made of the abovementioned disinfecting machines and tanks. In case of flowage, the liquid must be disinfected at the places where flowage occurs in order to prevent the spreading of infection.

#### DISEASE-CARRIER CONTROL (INSECTICIDES AND REPELLENTS)

In the opinion of foreign experts, one of the likely means of a bacteriological attack, as pointed out earlier, is the occurrence of infection carriers. For examination of environmental disease carriers, DDT preparations and hexachloran, as well as their recently synthesized derivatives, are widely used abroad (13). The recommended DDT derivatives are methoxychlorine and the so-called TDE (1,1-dichlor - 2,2 bis [parachlorophenyl] ethane).

The structural formula of methoxychlorine is as follows:



It is used against mosquito larvae and it is less toxic than DDT.

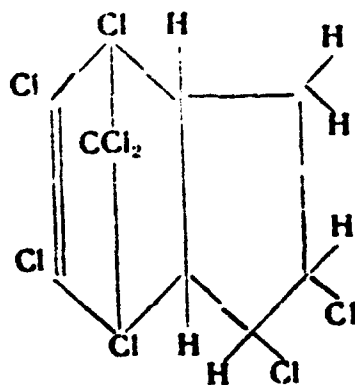
A recommended hexachlorane derivative is a gamma-isomer, referred to in foreign literature as gamma benzene hexachloride. Lindane is an American preparation containing at least 99% of gamma benzene hexachloride.

The advantages of this preparation, as compared to hexachlorane, are its greater effect on insects and its less offensive odor. Lindane is widely used in practical epidemiology and is manufactured in the U. S. as a concentrate containing 20% lindane, 7.5% emulsifier, up to 40% isoparaffin, and 32.5% alkylated naphthalene. It is a standard preparation used by the U. S. Armed Forces (13).

In addition to the mentioned substances, foreign sources refer to synthetic insecticides, such as: chlordane, dieldrin and derivatives of pyrethrin alkaloids obtained from pellitory of Spain.

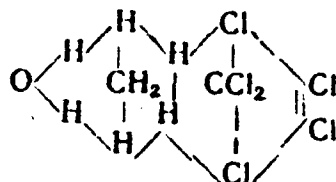
In the U.S.A., organic phosphorus compounds (parathion, malathion, diazinon, etc.) are used for pest control (13).

Chlordane ( $C_{10}H_6Cl_8$ ), also known as octachlorine, was synthesized in 1945. Chlordane is a viscous, amber colored liquid, boiling point  $157^{\circ}$ , and is easily dissolved in organic solutions. Its structural formula is:



Similar to lindane, chlordane is produced in the U.S. as a concentrate of 20% chlordane in refined kerosene.

Dieldrin ( $C_{12}H_8O_5$ ), and a similar preparation, aldrin, were synthesized in 1948. They are highly poisonous for insects and not without danger to man. Their structural formula is:



Dieldrin is manufactured as a concentrate containing 25% of the preparation mixed with refined oil products. It is used for disinfection of areas, roads, etc. Dieldrin is a standard insecticide in the use of the U. S. Armed Forces.

Pyrethrin and its alkaloid derivative - alletrin, synthesized in 1949, are regarded highly as insecticides by foreign experts.

Alletrin is a dl-2-allyl-4 hydroxy-3 methyl-2-cyclopentene monomer that is esterified with a mixture of cis-and trans-dl-chrysanthemic acids. It is also called an allyl homolog of cinerin I and synthetic pyrethrins. The effect of alletrin is slower as compared to pyrethrin.

The value of these preparations lies in their high toxicity, their quick effect on insects and their safety factor. Therefore, the American experts attach great importance to alletrin as an insect killer and have stepped up its production.

Pyrethrin and alletrin are frequently used in mixtures with certain substances that increase their poisonous effect. It has been established that such substances, in themselves insufficiently poisonous to insects, attain a high toxicity by addition of pyrethrins. This quality has been first discovered in sesame oil and later in a number of piperonyl compounds. They were named synergists or activators. The most practical of them is piperonyl butoxide.

For treatment of living areas, the American manuals recommend a solution of pyrethrin, piperonyl butoxide and DDT, while for open areas allethrin is used instead of pyrethrin. The addition of pyrethrins to the basic chemical insecticide - DDT- increases by several times the exterminating effect of the preparation. Thus, according to American sources, lice lose their blood-sucking property within 10 minutes after application of the combined insecticide, whereas the effect of a 10% dust starts after 1-2 hours. Special significance is attached to the addition of pyrethrins to the DDT preparations (0.2-0.3%) used for extermination of insects resistant to DDT (13).

An extract of pyrethrum is manufactured in the U.S.A. as a 20% concentrate in kerosene.

All the enumerated substances are basic insecticides used abroad against disease carriers and pest.

Side by side with insecticides, increased use has been made lately of repellents. The U.S. Armed Forces are provided with dimethylphthalate, 2-ethyl-1,3-hexanediol, indalone, dimethylcarbamate, benzyl-benzoate, butylacetanilide and others; they are used either independently or in various combinations with each other.

According to American researchers, identical repellents have different effects on different insects. Attempts were therefore made to establish a mixture of such substances so as to produce the widest possible range of application against a variety of arthropoda. As a result of a study of various combinations of repellents, a standard mixture, known in the U.S. as "6-2-2" was selected. This mixture contains 6 parts of dimethylphthalate, 2 parts of 2-ethyl-1,3 hexanediol and 2 parts of indalone. Lately, propylsuccinamate and dimethylcarbamate have been used instead of indalone.

During the Korean war, the American Army used as a repellent the mixture M-1960, which is composed of N-butylacetanilide, benzylbenzoate, 2-butyl-2-ethyl-1,3-propanediol and an emulgator<sup>1</sup> (13).

<sup>1</sup> There is no information on the proportions of the ingredients in this preparation.

When applied to clothes, this mixture preserved its protective properties during a full week of uninterrupted wearing in a dry, rainless weather. It was further established that some of the repellents, such as benzylbenzoate and diphenylcarbamate, preserve their protective properties even after several washings of the treated linen. According to recently expressed views of the American military experts, treatment of clothes with repellents is the most practical method of direct protection of armed forces in the field.

Sprayers of different systems and constructions are used in the treatment of surface clothes, linen, etc. with insecticides and repellents. For treatment of clothes, bed linen and living quarters, dusters (hand piston sprayers) and aerosol bombs are used. Dust containing insecticides is either blown under the collar, between the body and the underwear, or is applied to the inner side of linen and clothes. The dust is made of 10% DDT or 1% lindane on pyrophyllite or talc, with the addition of 0,2% pyrethrine, 1% piperonyl butoxide and 0.25% isopropyl-cresol. In the army, DDT dust is primarily applied against lice.

In order to form aerosols, a mixture of insecticides with freon is used in aerosol bombs. For treatment of small areas, portable sprayers equipped with a hand pump are also used in the American army. A solution of the insecticide in an organic solvent or in water emulsion is poured into the tank of the sprayer. Of great practical value are "wetting" powders, prepared by mixing of the insecticide with easily moistened inert powders. When mixed with water, these powders produce suspended matter which is introduced into the sprayers.

There are on sale in the U.S.A. wetting powders containing between 50-75% of industrial DDT. The main advantage of using wetting powders for disinfection, as compared with the use of emulsified concentrate and oil solutions, indicated in the American manuals (13), is that of the active ingredients of the former which are more concentrated and can be carried in dry form. They are fire-resistant and present no problems with regard to solvents, since water is usually readily available. They can also be mixed with a dry base and, if necessary, can be used in dust form. Among the disadvantages of wetting powders, one is the necessity of frequent mixing so as to

preserve the uniformity of suspended matter, and another disadvantage is the rubbing off of pinions in the pump of the mechanic sprayer.

In addition to hand sprayers, the American Army also uses powerful motor-driven spraying equipment. Such equipment is used primarily in treatment of settled areas, roads and large installations. A traveling hydraulic sprayer, which is a standard equipment in the use of the American Army, is mounted on a trailer and is equipped with a piston pump driven by an air-cooled gasoline engine. The sprinkling mechanism is equipped with four disks with openings of 1.6 to 2.7 mm. in diameter. The feeding of the spraying substance is carried out through a suction pipe 19 mm. in diameter. The pump discharge is no less than 6 liters per minute at a pressure of 21 kg/cm<sup>2</sup>. At this pressure the droplets that come out of the sprayer are smashed into very fine particles which are airborne for a short while. Under favorable weather conditions, the spray can attain a width of 180 m. (13).

According to calculations of a Florida laboratory, spraying at this width at a pump discharge of 6 liters per minute of 5% solution of disinfectant, will provide distribution of 130 g. of insecticide per 1 hectare at a speed of 18 km. per hour. If necessary, the dosage can be increased or reduced by regulating the speed of the vehicle.

Motor driven hydraulic sprayers are also used for spraying of orchards, trees and sowing areas.

Besides the hydraulic sprayers, there are in use in the U.S.A. motor driven pneumatic sprayers equipped with air compressors which provide the pressure for ejection of the liquid. One of such sprayers is the apparatus constructed by Hasman. Its capacity is 0.25 m<sup>3</sup> per minute at a pressure of 1.4 kg/cm<sup>2</sup>. The compressor, equipped with a rubber hose, is connected to a 19 liter capacity tank containing the insecticide (13).

The tank is equipped with a hose, a rising pipe extending down to the bottom and a regulating valve. The pressure is controlled by a manometer built into the tank cover. The sprayer head has six openings and is attached to a copper tube which is the nozzle of the hose.

Certain insecticide sprayers used in the U.S.A. are



built on the principle of heat generators. The spraying solution is either warmed up in these generators and evaporates in the same manner as gas disinfectants, or is carried by the fast moving stream of hot air, mixes with it and disperses in the open air. It is also recommended to use for such purposes the exhaust gas of the engine. A Venturi tube is attached to the exhaust pipe. The insecticide flows into the narrow part of the pipe where it is warmed up, then disperses as it expands on crossing the widened part of the pipe. The droplets that are formed in spraying with heat generators are very small, and therefore the mist which is created, stays long in the air.

For treatment of large areas the most practical method is the use of airplanes and helicopters. The airplanes are equipped with special installations similar to those used for sprinkling of toxic substances.

The American manuals on disinsection contain a description of a so-called longeron installation for spraying of insecticide solutions from airplanes. Holes are bored through the longerons and tubes, connected with tanks of different capacities, are inserted. The liquid flows from the tanks under pressure into the tubes and disperses with the air currents. Surplus pressure in the tanks is generated by a pump which is installed under the fuselage and is activated by the wind (13).

Thus, considerable attention is given in the U.S. to the problems of extermination of harmful insects. According to American experts, all the insecticides and repellents mentioned in this section can also be used against warfare agents of the disease-carrier type.

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## ORGANIZATION OF ANTI-BACTERIOLOGICAL DEFENSE MEASURES

In preceding sections, data from foreign literature was cited on the role and significance of a number of fundamental measures (detection, individual and collective defense methods and disinfection) which have been adopted in the United States of America. In the present chapter a specific analysis is made of an entire complex of more important defense measures which have been recommended, beginning at the moment the bacteriological weapons have been used by the enemy and concluding with the eradication of the results of the attack.

One must make the reservation that many extremely significant organization problems relating to anti-bacteriological defense are not cleared up in this section; this is due to the absence or extreme scantiness of concrete data on these problems in the American press. The basic foreign data in the literature are cited below.

Inasmuch as bacteriological, atomic and chemical weapons can most probably be applied from the air (in the form of bombs, shells, containers, etc.), it is assumed that in the majority of instances the moment when they are applied will be preceded by an alert signal supplied by information of airforce antiaircraft defense facilities. After this alert is sounded, the population of the cities (inhabited centers) will be required to leave their apartments and to take cover in the shelters.

The fact that bacteriological weapons are employed by an adversary can be detected by observation posts of the civil defense ground-spotter corps on the basis of visual sightings (for signs of bacteriological weapons having been used, see Chapter V). Full confirmation of such suspicion can be obtained only through a laboratory study of air and water samples, as well as other objects occurring in the surrounding environment. The task of gathering and collecting samples rests with the local

communes of the bacteriological reconnaissance organized from among volunteer members of the civil defense service as well as with mobile laboratories of the State Public Health Services and special mobile detachments comprising district diagnostic laboratories of the State civil defense service. The duties of these mobile laboratories and detachments consist, along with the collection of samples, also in the execution of the simplest and fastest analyses, especially in the area of non-specific indication and in the routing of samples to the nearest stationary laboratory. Further laboratory studies aimed at identifying the inducer are performed in district diagnostic laboratories, where preliminary data is obtained, in the State control laboratories, in local bacteriological laboratories of the State Public Health Service, in hospital laboratories, as well as in clinics and research-scientific institutions [1,2].

It is assumed that in a number of cases, laboratory studies involving indication will permit one to obtain an answer as to the identity of the agent within a relatively short time, i.e. prior to manifestation of sickness cases. At the same time, however, it should be taken into consideration that identification of a series of agents (especially rickettsia and viruses) requires a considerable amount of time. In these instances (as well as in those cases in which a diversity of bacteriological weapons is applied) the kind of agent will be ascertained on the basis of clinical and laboratory diagnosis of the first manifested cases of infectious diseases [1].

Immediately after establishment of the fact regarding application of bacteriological weapons by the enemy and sounding of the appropriate alert, measures designed for the direct protection of the population (or troops) from the bacteriological attack are put into effect. Persons caught in the street should don an individual frameless gas mask (evidently, a model adopted for use by the civilian population - A. F.). If this is not readily available, it is recommended to cover the nose and mouth with a doubly folded handkerchief, scarf or any other clothing object so as to avoid inhalation of the bacteriological aerosol [2]. It is necessary to don special protective garments (best of all anti-gas ones), and in case of their absence fasten tightly all the buttons of normal clothing objects, tie them around the wrists and ankles and put on gloves [3]. It is recommended, whenever possible, to take cover in buildings, porches, closed automobiles, or any other sheltered place [2].

In the USA armed forces, customary military gas masks and anti-gas protective garments are used as individual defense means against bacteriological weapons [3].

The gas mask and the protective garments can be taken off

only after emergence from the infected district and performance of individual disinfection [3].

The best means for collective protection are shelters equipped with antigas facilities; they provide protection against bacteriological weapons without having in them gas masks and protective clothing. Relative protection from a bacteriological cloud and spray is afforded by ordinary accommodations having tightly closed windows and doors. Obviously, however, they can not ensure safety at a high concentration of, and prolonged exposure to aerosol; therefore when various buildings, fortified structures, and dugouts, devoid of antigas facilities, are used as shelters, it is necessary to put on gas masks and protective clothings (if they are available).

Troops under field conditions employ regular means of collective defense [1].

As soon as circumstances will only permit, work aimed at liquidating the bacteriological attack results should be started. A list of these measures has not been formulated exactly in American manuals. However, generalizing separate data, cited in the literature, the conclusion can be drawn that it includes the following: limits determination of the infection nucleus, organization of quarantine, performance of special prophylaxis and immunization among the infected, exposure of the sick and their isolation, maintenance of evacuated persons requiring medical attention, as well as measures involving disinfection and sanitary-hygienic supervision.

Determining the area of the infected district is carried out as soon as possible after ascertainment of the fact that a bacteriological attack was committed. The district boundaries are marked with identifying signs and the access to it is halted. According to American directives [1] the period during which entry to the contaminated territory is denied will be, evidently, a short one, since the area in question is subjected very rapidly to natural disinfection, being mostly under the influence of solar radiation.

We were unsuccessful in finding in the available literature concrete indications regarding the character of the hygienic restrictive measures which would be adopted in the contaminated district. Considering, however, that in a bacteriological attack, public health organs will be charged with "the execution of measures involving the establishment of quarantine" [1,4], it can be assumed that quarantine, evidently, is not envisaged in all of the cases. It is probable that in non-contagious infections, the hygienic methods will be limited only to the isolation of the sick.

After ascertaining the kind of **agent** employed, specific protective measures against the bacteriological weapon are put into effect.

All the persons found within the territory of the contaminated district are subjected to active immunization against infection, the **agent** of which is applied as an antagonist. In addition to that, special seroprophylaxis of the expected disease among the most important category of the population [troops; in the defense set-up is performed [whenever the appropriate serum is available]. Execution of special prophylaxis with antibiotics and chemopreparations is undertaken only among limited population and troops contingents.

All of these measures, aimed at preventing the development and propagation of infectious diseases should be accomplished by local forces of public health organs [1]. They are entrusted with exposure and isolation work of infected sick people. The ill uncovered in this fashion are sent to hospitals and clinics where diagnosis of their disease is made and treatment is conducted [1]. Before placement in medical institutions, the ill and sick-suspects are subjected to sanitary treatment consisting of disinfection of clothes and underwear [2].

According to official recommendations [3], placement of wounded and sick under conditions of "an extraordinary state" [atomic, chemical, bacteriological attack] will be conducted not only in hospitals operating during peace time, but also in those functioning during wartime.

Medical institutions of neighbouring states are assumed to be employed for hospitalization of wounded and sick from the district having suffered the attack.

Moreover, the medical service of industrial enterprises can, evidently also be included in the exposure and isolation work of the infected sick. Its function at the present time is restricted to control of maintaining sanitary-hygienic standards; however, during wartime this service, according to the requirements of civil defense organs, can be strengthened considerably. This will ensure the creation at every large enterprise of a medical center which, when equipped properly, can serve as a temporary hospital [5].

We did not encounter in foreign literature more precise indications regarding the medical-evacuation maintenance of the infected sick under conditions of bacteriological warfare. As for widely illuminated cases in American literature regarding organization of medical-evacuation provision under conditions of an atomic attack, they must, obviously, be examined separately, since they do not envisage maintenance of an anti-epidemic regime on evacuation stages and routes.

The entire contaminated district together with buildings, defense works, transports, and other equipment contained in it are subjected to compulsory disinfection, while people trapped in the area undergo sanitary treatment.

The scope of sanitary treatment depends on the make up of the tactical situation and available means. It is considered that at the first possibility all persons having been exposed to the bacteriological weapon should undergo complete sanitary treatment, changing their contaminated clothes and underwear [2,3]. Special washing centers [1], in the organization of which USA army personnel and equipment take part are developed for this purpose [6].

Taking into account the enormous scope of measures concerned with disinfection, the latter is assumed to be performed only in small sections of the affected locality as well as in structures which are of considerable significance to the war effort. It is assumed that the following simple methods will be applied for widespread disinfection: mechanical cleaning, drying, ventilation, solar irradiation which will be carried through until the identity of the bacteriological weapon is ascertained. Stricter disinfection methods are recommended should it turn out that spore shapes were applied.

The use of bleaching powder is recommended for the disinfection of buildings, roads, and streets, as well as for the treatment of land plots and fields. Large continuous surfaces including the following: stone and brick fences, walls of buildings, solid surfaces of roads and streets, tile and concrete floors, etc. - are disinfected by simple water washings from hoses [5]; through the utilization of disinfecting agents (a hypochlorite solution and others) with subsequent hose washings, and with hot steam (from steam kettles). Disinfection of interior sites can be carried out by simple washing of floors and walls, ventilation, and sun drying of rugs, stair carpets, and even furniture [5].

Benzene and acetylene burners are sometimes employed for the disinfection of some forms of metallic equipment and non-flammable objects.

All of these measures comprise the responsibilities of civil defense sanitary services; it is assumed that USA armed forces participate in its activity.

The entire personnel of civil defense detachments working on bacteriological weapons detection and execution of measures concerned with disinfection is provided with gas masks and protective clothes. After disinfection of the contaminated district, the people undergo a complete treatment and receive clean underwear, while their clothes are disinfected or destroyed [2].

Food products and water contained in the district affected by bacteriological weapons are suspected to be infected, and their consumption is prohibited pending special permission.

The entire water designated for communal needs is disinfected through chlorination, which is considered as an almost universal method for its purification. Moreover, it is assumed, that the intensity of chlorination in water pipes should not be lower than 1 : 1,000,000 of residual chlorine after a 15 minute contact period [5]. In case it is impossible to organize water chlorination, it should be subjected to compulsory boiling.

Frequent bacteriological water analyses [several times per day] should be performed on large water pipes [water springs], in order to ensure continuous water control [5].

Under field conditions, water supply is provided by military water works in which, besides chlorination, the water is also filtered. In order to provide water for small military groups, the so-called Lister bag is employed which is adopted by the American army for water supply. Moreover, water for disinfection can be subjected to boiling. Inasmuch as spore forms of microbes yield poorly to customary disinfection methods, in cases of bacteriological attack, pending special permission, only boiled water should be consumed [3].

All food products, contaminated or suspected to be infected, are destroyed or subjected to disinfection [cooking, boiling] [3], as determined by local authorities. In order to supply the population of the contaminated district with food, a special service of communal care is created in the civil defense system [5]. It is considered that control involving execution of normal sanitary-hygienic measures in the contaminated district should be strengthened sharply. However, in the opinion of American specialists these measures should not differ, in essence, from similar ones put into effect in time of naturally arising epidemics. All responsibility for their execution rests with the sanitary sections of the State public health departments.



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